Dear Dr. McCulloch,

We very much thank you and the reviewers for their comments and suggestions. We feel that their input has greatly benefitted the manuscript. Accordingly, we have rewritten the manuscript to address reviewer concerns and, where necessary or suggested, included additional discussion. We feel that the manuscript's clarity was much improved by this. We present our work, "Axial and Radial Forces of Cross-bridges Depend on Lattice Spacing", to PLoS Computational Biology for resubmission with edits. We have addressed reviewer comments in the paper and comment-by-comment in text that follows. Reviewer comments are in bold type face and our responses are in standard type face.

You asked as well for cover art. We have uploaded an image that is inspired by (and derived from) our simulations. We have also included additional supplemental information: two figures to address specific review comments.

Sincere thanks,
C David Williams
PhD Candidate
University of Washington

Reviewer #1 (Remarks for the Author):

Cross-bridge models have for many years become more complex by increasing the number of biochemical states, without becoming more realistic by incorporating new structural and mechanical data at the molecular level. Williams et al. describe a new cross-bridge model that is a major step in the right direction. Their primary, long-term motivation is to investigate the Frank-Starling mechanism of the heart by predicting the influence of filament lattice spacing on cardiac muscle mechanics. A new cross-bridge model is a prerequisite for such an investigation because existing cross-bridge models do not incorporate the distance between thick and thin filaments as a variable. Not only do the authors introduce a new and more realistic model, the also identify a simpler and computationally more tractable version that captures the key features of the more complex model, but will speed up their future simulations.

Major concerns

The manuscript is missing any discussion of how the model kinetics have been adapted for cardiac myosin, and what cardiac myosin (human or small rodent, alpha- or beta-MHC isoform) is the target. What parameters in the model would be altered to allow simulations of these various myosins, primarily compliance or kinetics, or is it necessary for both to co-vary?

Adapting our model to target specific types of myosin, or their mutants, has the potential to be a rich area for further study, and we are grateful to the reviewer for this chance to address it. In the current manuscript, we sought a more general model that replicates the geometric and kinetic characteristics that are shared between cardiac, skeletal, and insect myosins. A complicating factor, moving towards specific myosin models, is that many of the parameters we need are only available from x-ray crystallographic studies (e.g. Rayment et al. 1993), which are not yet available for all myosins. Some data, such as extensibility of various regions, which would assist in targeting our models, does not exist for any myosin. Once such parameters are available, we intend to test to what extent various myosin types are differentiated by compliance or geometry, and to what extent they are differentiated by kinetics.

We suggest that changing geometry and compliance may be sufficient. The kinetics of our models are functions of the free energies of the cross-bridges, free energies which are functions of the cross-bridges' stiffness and rest values. Changing the compliance or rest value of any part of the cross-bridge will have a corresponding effect on the cross-bridge's energies and thus on the cross-bridge's kinetics. It would bolster our approach to the kinetics of the cross-bridge if such modeling efforts don't require a change in kinetics.

We have added discussion of the model's general target, and of the relationship between kinetics and spring values, to the introduction to the section on kinetics, on pages 9 and 10.

It would seem appropriate to briefly consider in discussion the possibility that disease-related mutations in cardiac myosin might have their major effects through changes in cross-bridge compliance. For example, see two papers from Kraft and colleagues (Köhler, J., G. Winkler, I. Schulte, T. Scholz, W. McKenna, B. Brenner, and T. Kraft. 2002. Mutation of the myosin converter domain alters cross-bridge elasticity. Proc. Natl. Acad. Sci. USA 99:3557-3562; Seebohm, B., F. Matinmehr, J. Köhler, A. Francino, F. Navarro-Lopez, A. Perrot, C. Ozcelik, W. J. McKenna, B. Brenner, and T. Kraft. 2009. Cardiomyopathy mutations reveal variable region of myosin converter as major element of cross-bridge compliance. Biophys. J. 97:806-824). Thin filament mutant's effects on compliance might also be important in the broader model, but that is beyond the scope of the current manuscript. The model may ultimately provide important insight into cardiac disease mechanisms.

This is an exciting point that we are very happy to include in the discussion. We have added a new paragraph to the discussion on page 7 which addresses the possibility of using data on changes in compliance that occur at the single myosin level in disease states to trace the emergence of disease phenomena at the half-sarcomere level. We very much thank the reviewer for raising this interesting point.

What are the implications for shortening velocity when the new models are missing the asymmetric cross-bridge detachment rate that were built into the 1sXB model? Williams' approach that avoids discontinuous functions is certainly more appealing, but is it as successful as Huxley's at explaining such a wide variety of physiological phenomena?

The effects of our multi-spring models on simulations of shortening velocity are now addressed in a new paragraph in the discussion on page 6. We treat the effects of both changes in step size and detachment rate constants as lattice spacing changes. While it is not currently possible to foresee the full range of effects our modifications will have until such cross-bridges are embedded in a model of interacting filaments, we have extended our extrapolation. One interpretation of our results is that the changes in step size and detachment rates we see as lattice spacing changes should be able to account for the changes in shortening velocity that have been observed with changes in lattice spacing and sarcomere length.

Williams introduces arbitrary "efficiency factors" for the free energy of the attached states (page 10). What is the sensitivity of the model to the values used?

The efficiency factors used on page 10 are the values used in Tanner et al. (2007) and are derived from the work of Pate and Cooke (1989). They are governed by the drop in free energy from the unbound state to the loosely bound state and from the loosely bound state to the strongly bound state. They are the fraction of free energy liberated by the hydrolysis cycle that is available for use in a given state. We have revised the discussion of efficiency factors on page 10 to make this clearer.

Minor concerns

Page 2, line 11 from bottom: omit use of 1980a,b for Schoenberg references. These designations are not included in the reference list.

Thank you, we have corrected the formatting.

Do the authors anticipate that their modeling with variable lattice spacing will help to resolve experimental differences and conclusions between laboratories on the importance of lattice spacing for the Frank-Starling mechanism?

Yes, when incorporated into a lattice of thick and thin filaments, these two-dimensional cross-bridges will naively incorporate the effects of lattice spacing on axial force generation. Thus as lattice as lattice spacing changes, we can explore cooperativity mechanisms that depend on lattice spacing and that influence the development of axial and radial forces generated by the half-sarcomere. Such future work, while beyond the scope of the current paper, is one of the more exciting applications of such two-dimensional cross-bridge models.

What is meant by "tuning" in the legend for Fig. 6? This isn't explained in the text, and isn't obvious in the figure.

We thank the reviewer for drawing our attention to this term. We intended to say only that the 4sXB and 2sXB models produce different step sizes, even if they both have a local maximum step size at a given lattice spacing with decreasing step size as lattice spacing diverges from that lattice spacing. The caption of Figure 6 has been updated to reflect this.

Fig. 3 is overly cluttered with redundant text and labels. Fig. 5 could be similarly simplified.

This is a very accurate statement; the redundant labels on Figure 3 and Figure 5 have been removed.

Fig. 4 C, D vertical axis begins and ends at 32 nm.

Thank you, the axes have been corrected on Figure 4.

Reviewer #2 (Remarks for the Author):

The goal of the present study was to develop multi-spring cross-bridge models and use these models to investigate how myofilament lattice spacing affects cross-bridge kinetics and force generation (both axial and radial forces). Most current geometric models depict a cross-bridge as a linear spring oriented along the myofilament axial direction. This is clearly inconsistent with the structural data and also with experimentally established observations that cross-bridges exert both axial and radial forces and radial forces are significant. Thus, a more realistic cross-bridge geometric model is desirable and interpretations of experimental data using such a model are likely to provide new insights. However, there are several concerns regarding the manuscript.

(1) It is not clear whether a multi-spring model is required for the observed effects of lattice spacing. Would similar results be obtained by simply orienting a single spring at a specified rest angle and rest length? It appears that this simple modification of the conventional single spring model will yield similar results - existence of radial force, dependence of cross-bridge force and free energy (and consequently of cross-bridge kinetics) on both axial offset and lattice spacing. It is acknowledged that the 4sXB model better mimics the real cross-bridge structure, but the 2sXB model could be tuned to yield similar results. It is desirable to know whether a torsional spring is mandatory or whether an angled linear spring (force-generator) would suffice.

We thank the reviewer for raising this point as it was something we considered in the early stages of model design. Let us take such a cross-bridge to be composed of a single linear spring attached at a 45 degree angle to the thick filament. For the cross-bridge to be composed of a single spring, the angle which it makes with the thick filament must be fixed or must rotate freely, a resistance to rotation would be the use of a torsional spring.

In the case where the angle is fixed, the translation of bound cross-bridge heads becomes problematic. The bound cross-bridge head that is held at a fixed angle cannot move in the axial direction without changing its length. This length change, occurring at an angle to both the thick and thin filaments, requires a change in the radial spacing between the respective thick and thin filament nodes. Our example 45 degree cross-bridge would require a 1nm change in local lattice spacing for every 1nm axial translation. This large deformation in lattice spacing is energetically unfeasible and wreaks havoc on transition rate schemes.

In the case where the angle is allowed to change freely, making the thick filament cross-bridge attachment point a freely rotating joint, the cross-bridge is unlikely to perform any net work. The freely rotating cross-bridge is equally likely to bind at any point with the same radius to the thick filament attachment point. This results in cross-bridges binding both forward of and reverse of the axial location of their thick filament attachment points, and thus undergoing power strokes that cause both contractive and expansive forces. Thus, without further restrictions there is no mechanism to "rectify" the work done by the cross-bridge such that it is biased in the forward direction. As we can use neither the case where the angle between the cross-bridge and the thick filament is fixed, nor the case where the angle is freely rotating, the use of an angular spring is necessary.

(2) Some of the results have to be the way they are almost by definition. For example, cross-bridge attachment rate constant decreases and detachment rate constant increases as lattice spacing increases. This is a direct consequence of the prescribed dependence of these rate constants on cross-bridge free energy, which increase as the lattice spacing is altered from its rest state because of the prescribed cross-bridge geometry (any angled force generator will do this). Similar comments apply to the existence of the radial force and its relative magnitude.

The reviewer raises a good point. These results are generally expected, although they have not been explicitly modeled as a mechanical system before. This paper serves as a demonstration that, given kinetics which operate in the radial dimension, dependence on lattice spacing and the production of radial forces inevitably emerge. These properties are intrinsic to the multi-dimensional cross-bridge. The phenomena we observe are bolstered by experimental observations, they do not challenge them.

The manuscript will be significantly strengthened if some of the model-based predictions (calculations) are compared with existing experimental data.

We know of no radial force data at the filament or cross-bridge level to which we can compare the current work. In future work, we will produce force predictions at the sarcomeric scale by embedding this model in a lattice of compliant filaments, as done in Tanner et al. (2007) and Campbell (2009). This future work, currently in preparation, will then allow comparison to experimental data such as Bagni et al (1994) and Brenner and Yu (1991).

(3) The presentation of the model calculations, results, and interpretations is quite confusing. The authors should make every attempt to improve the presentation and readability aspects of the manuscript. Here are some (not exhaustive) examples of this confusion.

(3.1) It is not clear as to what the prescribed quantities were and what was calculated or predicted by the model-based simulation. It would be very helpful to provide a schematic diagram illustrating the step-by-step model simulation protocol, beginning with the prescribed inputs and followed by sequential calculations of various variables. This will also clarify what the real model-based predictions are.

We thank the reviewer for suggesting such a figure. We agree that a visualization of the model's process is useful and have created and added a new supplementary figure to that end.

(3.2) Figs. 3 and 5: A number of statements are made in the context of these figures that comment on the steepness of rise or the location or magnitude of the minimum of a given quantity with respect to lattice spacing or axial offset. The format of these figures does not allow the reader to readily appreciate these points. It would be much better to present these data as line graphs (similar to Fig. 2), with multiple curves within a given panel corresponding to various lattice spacing (e.g., 2 lattice spacing: rest, above rest, and below rest).

We agree with the reviewer that contour plots, while displaying a complete picture, frequently make comparison of precise values between different lattice spacings difficult. We have created a figure similar to that suggested and have included it as a supplement to the manuscript.

(3.3) Page 3, definition of axial offset: "The axial offset of cross-bridge property is the distance from thick filament attachment site to the property's extreme value or point of inflection at a given lattice spacing" - this is a very unclear statement; perhaps this can be better explained in the context of a figure. (Note: The suggested figure format change in #3.2 may help here.)

We thank the reviewer for this comment and have revised the manuscript accordingly. An explanatory example of axial offset has been included at the bottom of page 3, below a revised version of its definition.

(3.4) Page 4, cross-bridge force and lattice spacing: "The axial and radial forces at a given axial offset increase as lattice spacing grows larger" - Fig. 4 indicates that the total cross-bridge force has a minimum at the rest lattice spacing and it increases above and below this rest spacing (compressive in one direction and expansive in other). Thus, it is not clear how the stated monotonic relationship between axial (radial) force and lattice spacing can hold.

This is an excellent point; in this description we had switched from using absolute values, talking about the magnitude of a property, to using vectors to describe the axial and radial forces. That is, axial and radial forces do increase as lattice spacing grows larger, starting out as very negative values, becoming less negative, passing through zero, and growing more positive. We have revised the relevant section on page 4 to increase clarity, moving to the terms expansive and compressive.

(3.5) Page 5, Discussion, two key features of 4sXB and 2sXB: The first key result talks about the dependence of step size on lattice spacing. The results section does not present any data regarding this issue. There is a paragraph in the Discussion section about this - some of this material should have been in the results section. The definition of the step size and the protocol for calculating it in the model simulation is not clear - again, a schematic figure may help. It is stated on page 6 that "This additional set of springs ... and causes the 4sXB model's step size to depend less strongly on the magnitude of change in lattice spacing (Fig 6)." However, Fig. 6 shows just the opposite - over the identical range of lattice spacing, the maximum variation of step size is about 4 nm for 4sXB (Fig. 6, left panel) and about 2 nm for 2sXB (Fig. 6, right panel).

We thank the reviewer for drawing our attention to this issue. The discussion subsection on step size has been rewritten on page 6 and now includes discussion of how changes in step size relate to trends in unloaded shortening velocity. A corresponding results section has been added to page 4. The step size comparison on page 6 has been corrected.

(3.6) Page 6, 2sXB vs. 4sXB: That the 2sXB model produces results similar to those produced by 4sXB is not surprising - again, this is primarily by design; the 2sXB model parameters were adjusted to yield pre- and post-power stroke tip location and kinetics to match the 4sXB model (page 7).

This is a very good point and signals that we should clarify the similarities between the 4sXB and 2sXB models. We have rewritten this section on pages 6-7 to put more emphasis on the relevant point: that the 2sXB model is capable of imitating the 4sXB model. We feel that the key point here is not that the properties of the models are fortuitously similar, but that the 2sXB model (a vastly more computationally efficient system than the 4sXB model) is capable of being tuned so that it replicates the 4sXB model's results. This similarity points to the key feature needed to study the effects of lattice spacing and radial forces being the presence of a torsional spring driving a lever arm, rather than the specific system that is the 4sXB model.

(3.7) Page 6: "... the 2sXB's axial force is more steeply dependent on lattice spacing (Fig. 5A, C). This pattern is reversed for radial forces..., where the 2sXB model's radial force is more dependent on lattice spacing ... (Fig. 5B, D)." This is a very confusing statement - it seems to indicate that 2sXB model forces (both axial and radial) are more sensitive to lattice spacing, so where is the reversal of the pattern?

We thank the reviewer for drawing our attention to this point. The section in question, which spans pages 6 and 7, now reads "the axial force produced by each model increases with lattice spacing, but that produced by the 4sXB does so more steeply (Fig. 5 A,C)."

(3.8) Page 7, line 2: "such as" instead of "such at."

Corrected, thank you.

(3.9) Page 8, calculation of lattice spacing: This discussion is quite unclear. It appears that lattice spacing and axial offset were the prescribed quantities. If so, what does the calculation of lattice spacing mean?

The section has been rewritten to be clearer. The goal of the section is to relate the values of lattice spacing used within the model to the values of lattice spacing that are used to present the model's results and which are more common in literature. Specifically, the model uses the distance from the face of one thick filament to the face of an adjacent thin filament to set the lattice spacing. This is the measurement that most easily permits calculations of cross-bridge length and force. However, this value is not commonly used in muscle literature while the d_{10} measurement is. The section presents information needed to convert from the value a model must use to the value more commonly seen.

(3.10) Page 9, calculation of spring lengths and angles: Again, the readability of this text can be greatly enhanced by illustrating these coordinates and distances in a schematic figure (perhaps, one of the existing figures).

The current figures cannot easily accommodate extensive additions of coordinates. We struggled with this issue, and instead chose to further clarify the text associated with the complex computation of lengths and angles.

(3.11) Table: Include parameters for the 1sXB model.

An excellent suggestion, we have done so, appending new rows to the table. These parameters are from Tanner et al. 2007.

(3.12) Fig. 3: Missing units for the Y-axis variables in panels C-H. Also, these are rate constants, not rates - please make these changes here and throughout the manuscript.

These changes have been incorporated throughout the manuscript.

(3.13) Fig. 4: Panels C and D: Confusing Y-axis scale - "32" appears twice. Also, it would be useful to include directions of compression and expansion for the axial and radial force components.

The Y-axis has been corrected and the directions of compressive and expansive forces are now indicated explicitly in the caption of figure 4.

Reviewer #3 (Remarks for the Author):

Review of 'Axial and Radial Forces of Cross-bridges Depend on Lattice Spacing'
C. David Williams, Michael Regnier, and Thomas Daniel

Summary

This manuscript describes development of a myosin crossbridge kinetic/mechanical model that accounts for putative geometric and structural arrangements in its formulation. By representing the crossbridge as a simplified system of two linear and two torsional springs, the authors are able to estimate the dependence of kinetic rates and force production on the two-dimensional location of a target actin binding site. A simplified model consisting of one linear and one torsional spring was also explored. The principal findings of the study are that (1) Lattice spacing (distance between myosin head and binding site along the radial axis of the muscle fiber) alters the myosin step size, or the distance traveled in the axial direction by a myosin head following power stroke, and (2) The formulated representation of myosin crossbridges produces combinations of radial and axial forces that appear consistent with those reported in the literature. It was also found that the simplified,
2-spring model captured most essential features of the more computationally expensive 4-spring model.

This topic is highly relevant to a number of lines of muscle research, and an analysis such as the one presented in this manuscript is long overdue considering the critical role it is thought to have in the regulation of muscle contraction. Many have proposed that the length dependence of calcium sensitivity in muscle is mediated by lattice spacing, a mechanism which would operate by altering the probability of crossbridge binding. This hypothesis, and the evidence supporting it, remains controversial. The present study lays the groundwork for quantitative investigation and analysis of previous results and therefore could shed light on a long-standing question. While the direct conclusions of this work may not be compelling to a wide audience, it is the opinion of this reviewer that it is a critical step forward and has the potential to be impacting and useful to the field as it is applied toward answering these important

questions.

Major Comments

My main concerns are centered around clarity in the presentation of certain portions of the methods and results. The standard for this journal is that the methods should be sufficiently detailed to allow other workers to reproduce reported results. This is especially important since the value of this work will only fully emerge as other studies make use of the formalism set forth in the methods. The following elements must be clarified in order to be acceptable:

1. Binding rate calculation - In previous work (Tanner et al. 2007 and Daniel et al. 1998) crossbridge attachment rate (r12) was directly proportional to the value of the PDF describing tethered diffusion of the myosin head for a given crossbridge distortion. The present work uses the same PDF, but in a much different way. It therefore needs more careful description and justification than given at present. The description of perturbing the distortion of each spring element is very difficult to follow due to mixing of algorithmic and theoretical steps. This should be resolved in such a way that both aspects are clear.

We thank the reviewer for pointing this out. In previous work the r12 transition rate constant was calculated using a combined calculation of diffusion of the spring representing the cross-bridge and a binding probability based on the distance of the cross-bridge tip from the nearest binding site. We retain this process, but split it into two steps: a diffusion step followed by a binding probability calculation. This split is necessary for the multi-spring cross-bridges as their geometry does not permit a simple diffusion step which may be integrated into the calculation of binding probability.

We have revised the section on “Binding rate calculation” on pages 10 and 11, separating the description of the algorithm and the mathematical details, for increased clarity. We have given special attention to the link between prior binding rate constants and our own at the top of page 11.

Also, there is no clear justification provided for the 'second step' of thin filament binding that is new to this work, wherein probability of attachment is proportional to exp(-d), where d is the displacement of the head to the binding site. Because this is a departure from previous formulations, and because its accuracy will determine the predictive relevance of the model, it must be justified very explicitly.

We appreciate this comment. As above, this technique is taken from previous work, but is spit into two steps to accommodate the more complex diffusion calculation needed by the multi-spring cross-bridges. This has been revised as above on page 11.

Some additional details regarding simulation of the ensemble of crossbridges used to estimate r12 should be included. What was the 'given time step' used to evaluate binding events? Were the results sensitive to this number?

We thank the reviewer for raising this point. Our chosen time step was 1 ms. We have revised the text to reflect this, primarily in our description of “Binding rate calculation” on page 11. As in the work of Daniel et al (1998) and Tanner et al (2007), this time step is included as an implicit scaling factor in the kinetic rate constants, making the results independent of the chosen time step.

2. Detachment rate - It is not clear what displacements (lengths and angles) are being used in the calculation of the energy (U1) the bridge would have following detachment. In the attached state, the energy seems straightforwardly defined by the location of the binding site. What happens following detachment is not intuitively clear to this reviewer, but certainly some assumptions have been made by the authors. These must be more explicitly stated. The reverse attachment rate (r21) is affected by this same issue - there is some mention of re-treating an ensemble of crossbridges to make this calculation, but again it does not read clearly enough for someone else to reproduce the work.

As shown in the equations of the "Free energy in each state" subsection of the description of model kinetics, the free energy of the detached cross-bridge is set to a reference point of 0. As in prior work, the detached cross-bridge is assumed to relax to an unstressed state. As the reviewer points out, this process was not easy to find when reading the section on detachment rates. We have rewritten the relevant section on "Power stroke and detachment rates" at the bottom of page 11 and inserted appropriate references to this information in the following section.

3. The implications of crossbridge geometry on the balance of axial and radial forces is clearly an important part of this work. At several points (particularly in Figs 4 and 5 and throughout the text) when force is being discussed, it is not clear whether the force refers to the pre- or post-powerstroke state. This needs to be clarified. Along these same lines, it seems as though the effects of lattice spacing (particularly the transitions from compressive to expansive highlighted in the text) could be different, depending on what state the bridge is in.

We appreciate the reviewer's point regarding our force description. All forces depicted are in the post-power stroke state and, throughout the manuscript, figure captions have been updated to reflect this. The reviewer is correct that the effects of lattice spacing vary somewhat with what state the cross-bridge occupies. While we focus our discussion on the post-power stroke state for reasons of clarity, the trends described in the paper apply to forces in both the pre- and post-power stroke states.

4. It seems like the implications of the work are not adequately discussed. Much of the results section merely recapitulates what was stated in the results section. The authors should build closer ties to experimental work by discussing not only which questions will be affected by the results, but also how.

The reviewer raises a very good point. We have re-written our discussion section on pages 5-7 to relate our results more directly to the field.

Minor Comments

1. It would be helpful if throughout the text the terms used to describe spatial components of force and movement were standardized. In my view the term 'direction of contraction' is ambiguous and should be replaced by something like 'parallel to the long axis of the myofilaments', which could be shortened subsequently to 'fiber axis' or similar. This would be less distracting, as the terms 'contraction' or 'shortening' are loaded with too many other connotations.

"The long axis of the myofilaments" is clearer and has been incorporated.

2. Page 2, bottom: The sentence referencing Schoenberg needs grammatical attention.

Thank you. It has been revised for greater clarity

3. Page 3, last sentence of the first paragraph: This notion of axial offset is confusing as presented here, and is described in the same language but more effectively later on. One of these instances should be removed as they are redundant.

We agree and have removed the first explanation.

4. Middle of page 3, directly following reference to Figure 2B: I'm reasonably certain that the description of results is backwards - it seems like multi-spring models are MORE likely to bind at small offsets.

We greatly thank the reviewer for noticing this. The offsets referred to were intended to be relative to cross-bridge's resting positions, not their axial offset from the thick filament attachment site. We have reworded the sentence in the middle of page 3 to remove the wording's similarity to axial offset.

5. Bottom of page 3: The term 'forward biasing' does not have clear meaning. The authors should consider more careful description of the term or the corresponding results. They are interesting, and perhaps should be emphasized a little more.

The term was ambiguous and has been removed from the section. A further discussion of issues related to changes in cross-bridge extension upon binding is now provided in the rewritten discussion section on step size on page 6.

6. Bottom of page 4: The idea that at larger lattice spacing bridges produce more force but are less likely to bind is repeated twice within a few lines. One of the instances should be removed.

We have removed one of the redundant instances and rewritten the whole section for clarity.

7. Check spelling of last word on page 4.

Thank you, we have corrected this.

8. Lattice effects on step size are not adequately discussed. The effects on force are already included by virtue of the geometric analysis, so what importance is the change

in step size? It could have an important application in understanding the dependence of maximum shortening velocity on sarcomere length. This should probably be mentioned in the discussion.

We thank the reviewer for bringing up this point. The discussion now contains a section, on page 6, addressing the possible ties between lattice spacing, step size, detachment rates, and unloaded shortening velocity.

9. Bottom of page 10: There are some critical typographical errors in formulae for P(x) (I'm fairly certain that 'k' appears when 'R' was meant in two of the terms) and p_12(d) (a minus sign is missing in front of d in the exponent).

We greatly thank the reviewer for pointing out this error. The equation was typeset without differentiating between k, the spring constant, and kappa, the Boltzmann constant. This error, and the missing minus sign, have been corrected and a subscript b has been added to kappa to further differentiate it.

10. The value of tau is discussed, but not listed.

We thank the reviewer for pointing out this omission. The value of tau is now included in the discussion.