Modeling residual force enhancement with generic cross-bridge models

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The interaction of actin and myosin through cross-bridges explains much of muscle behavior. However, some properties of muscle, such as residual force enhancement, cannot be explained by current cross-bridge models. There is ongoing debate whether conceptual cross-bridge models, as pioneered by Huxley (A.F. Huxley, Muscle structure and theories of contraction, Prog. Biophys. Biophys. Chem. 7 (1957) 255) could, if suitably modified, fit experimental data showing residual force enhancement. Here we prove that there are only two ways to explain residual force enhancement with these ‘traditional’ cross-bridge models: the first requires cross-bridges to become stuck on actin (the stuck cross-bridge model) while the second requires that cross-bridges that are pulled off beyond a critical strain enter a ‘new’ unbound state that leads to a new force-producing cycle (the multi-cycle model). Stuck cross-bridge models cannot fit the velocity and stretch amplitude dependence of residual force enhancement, while the multi-cycle models can. The results of this theoretical analysis demonstrate that current kinetic models of cross-bridge action cannot explain the experimentally observed residual force enhancement. Either cross-bridges in the force-enhanced state follow a different kinetic cycle than cross-bridges in a ‘normal’ force state, or the assumptions underlying traditional cross-bridge models must be violated during experiments that show residual force enhancement.

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1. Introduction

The work of A.F. Huxley has had a tremendous impact on the mathematical modeling of muscle. His classic paper of 1957 has shaped the majority of mathematical muscle models of the past 50 years. The leap of insight that led to the formulation of his model has not been matched by subsequent modifications. That is not to slight the work of those who have in turn reshaped the original conceptualization of the Huxley model to be thermodynamically consistent [20,22], and then consistent with biochemical and biophysical experiments (e.g. [13,37]). Recently, there has been cumulative evidence suggesting that these cross-bridge models may be incomplete.

Most of this evidence revolves around a particular feature of muscle, called residual or steady-state force enhancement. When a muscle (or muscle fiber) is stimulated isometrically, then subjected to a transient stretch and held isometric a second time, the second isometric force is higher than the first even when length effects are taken into account (e.g. [1,4,5,16,18]). This force enhancement does not occur in passively stretched muscle. The increase in force seems to be permanent – in that when activation is held as long as possible (on the order of 30s), the force enhancement persists, appearing to reach a stable non-zero value [1,19]. The magnitude of this long-lasting increase in isometric force (hereafter called force enhancement) seems to be independent of stretch speed but does depend on the magnitude of stretch [46,11,12,5]. These experiments are summarized in Fig. 1. Force enhancement occurs on the ascending, descending and plateau region of the force–length relationship [17,38,41]. Force enhancement appears to be only weakly activation dependent and independent of fatigue [6]. Early experiments showed a decrease in stiffness [46], while more recent evidence seems to point to a small increase in stiffness in the force-enhanced compared to the isometric reference state [28,16].

As a result of these observations, it has been suggested that the ‘long-lasting component of the stretch-induced force increase probably originates outside the cross-bridges’ [6] (similar conclusions are made in [12,10]). Instead, researchers have turned to additional calcium dependent proteins (such as titin) to explain force enhancement [11,12,27], or history dependence in the contractile proteins actin or myosin (e.g. [6,30]), or sarcomere inhomogeneity (e.g. [10,32]). But others still argue that cross-bridges may contribute to force enhancement [41,28]. To date, there has been no rigorous analysis to determine whether it is possible to explain force enhancement with a cross-bridge model, and (if it is) to determine what characteristics are necessary in such a model.

Here, we present a proof that if force enhancement is to be explained with a cross-bridge model, there are only two ways to do...
so. We argue that only one of these has the proper stretch amplitude and stretch velocity dependence. This type of model differs from the current understanding of cross-bridge kinetics in that it predicts as of yet unobserved behavior of the myosin cross-bridge. The proof of these general statements requires a general analysis of force-enhancement, as opposed to an approach involving a particular model with defined states and rate constants. Despite its generality, this approach leads to specific predictions that may be tested experimentally to determine whether the force of muscle contraction can be explained solely by the interaction of individual myosin heads with actin, or whether calcium-sensitive proteins (like titin), cooperativity between myosin heads, and/or sarcomere length (or other structural) non-uniformities contribute significantly to muscle force production following active stretch.

2. Two steady-states

From Fig. 1, we see that muscle exhibits different steady-state force values at the same average sarcomere length (depending on contraction history) and that the steady-state force value depends on stretch amplitude while being approximately independent of stretch velocity. We wish to test whether this force-enhancement can be explained by cross-bridges. Therefore, we assume that these different steady-state force values arise from different steady-state cross-bridge distributions. One consequence of this assumption is that the series of differential equations governing the chemical reactions of the cross-bridges must have the additional restriction of the law of detailed balance: $A_{ij} = A_{ji}e^{-\Delta G/RT}$. However, this relation tells us little, since we have no a priori knowledge of $\Delta G$, not knowing even if it is finite.

The elements of $A_{ij}$ are not completely arbitrary. Each non-diagonal element is positive, while each diagonal element is the negative sum of all of the other elements in that column. Physically, this is because the sum of the elements of $n$ must be specified, as it represents the total number of molecules (or total concentration of that molecule) in the closed system. Conventionally the vector $n$ is constrained to sum to 1.

At steady-state, the following equation must be satisfied:

$$A_{ij}n_j = 0.$$  

We have already argued that there must be one non-trivial solution to this equation, since there is at least one vector in the nullspace of $A_{ij}$. However, in order to have more than one steady-state, we must have at least one other linearly independent vector in the nullspace of $A_{ij}$.

We introduce the term ‘fully-connected’ to refer to any reaction network in which one state can potentially become any other state through a series of reactions; that is, there is a set of non-zero rate constants that connect any two states. We may then prove that a network with multiple steady-states, i.e. an $A_{ij}$ with a two or more dimensional nullspace, must consist of more than one separate sub-network.
2.1. The proof

We note that each diagonal element $A_{ii} \neq 0$, because otherwise the $j$th state is isolated and the network is not fully- interconnected. We also note that the ordering of the reaction matrix is arbitrary, as the behavior of the system is independent of the numbering of each element of $n$. If there are $s$ states, a two (or more) dimensional nullspace implies that some linear combination of the first $r$ rows equals the zero vector, where $r$ is an integer between 2 and $s - 1$. Since the ordering of the matrix is arbitrary, we can ensure that each coefficient in that linear combination is non-zero. Thus, we can write:

$$-\sum_{i=1}^{s} a_{ij} A_{ji} + A_{21} - a_2 \sum_{i=2}^{s} A_{2i} + A_{31} - a_2 A_{32} - a_2 A_{33} + \ldots + A_{s1} - a_s \sum_{i=s}^{s} A_{si} = 0 \quad (3)$$

$$+ a_2 A_{12} - a_2 \sum_{i=2}^{s} A_{2i} + A_{23} - a_2 A_{24} - a_2 A_{25} + \ldots + A_{s2} - a_s \sum_{i=s}^{s} A_{si} = 0 \quad (4)$$

$$+ a_3 A_{13} - a_3 A_{23} - a_3 \sum_{i=3}^{s} A_{3i} - a_3 A_{34} - a_3 A_{35} + \ldots + A_{s3} - a_s \sum_{i=s}^{s} A_{si} = 0 \quad (5)$$

$$+ \ldots + a_s A_{1s} - a_s A_{2s} - a_s A_{3s} - a_s A_{4s} + \ldots + A_{ss} - a_s \sum_{i=s}^{s} A_{si} = 0 \quad (6)$$

where the coefficients $a_i$ are arbitrary, non-zero numbers (note that we start with $a_1$, so that the subscript corresponds to the row it multiplies). We can rearrange these equations to a more manageable form:

$$(a_2 - 1)A_{12} + (a_3 - 1)A_{13} + \ldots + (a_s - 1)A_{1s} = \sum_{j=r+1}^{s} A_{ij} \quad (3)$$

$$(1 - a_2)A_{21} + (a_3 - a_2)A_{23} + \ldots + (a_s - a_2)A_{2s} = a_2 \sum_{j=r+1}^{s} A_{2j} \quad (4)$$

$$(1 - a_3)A_{31} + (a_2 - a_3)A_{32} + \ldots + (a_s - a_3)A_{3s} = a_3 \sum_{j=r+1}^{s} A_{3j} \quad (5)$$

$$\vdots$$

$$(1 - a_s)A_{s1} + (a_2 - a_s)A_{s2} + \ldots + (a_s - a_s)A_{ss} = a_s \sum_{j=r+1}^{s} A_{sj} \quad (6)$$

2.1.1. Each $a_i$ must be positive

Let us assume that one (or more) of these $a_i$’s is negative. For convenience (and without loss of generality) we assume $a_2 < 0$, or $a_2 = -\alpha_2^2$. Then, Eq. (4) is:

$$1 + \alpha_2^2 A_{21} + (a_3 + \alpha_2^2)A_{23} + \ldots + (a_s + \alpha_2^2)A_{2s} \leq 0,$$

where we have used the fact that each element $A_{ij}$ is greater or equal to zero. The left-hand side is less than or equal to zero.

This equation can only be true if at least one other $a_i$, say $a_i$, is less than or equal to $a_2$ (since at least one of the elements of $A_{ij}$ is non-zero, by the assumption that $A_{2j}$ is non-zero). Then, we apply the same process to Eq. (5) to show that there is another coefficient, say $a_i$, that is less than or equal to $a_2$. Applying this process iteratively to the subsequent rows, we find that for every $a_i$:

$$a_i < a_{i-1} \leq \ldots \leq a_s < a_{s-1} \leq \ldots \leq a_2 \leq 0.$$

When plugged into Eq. (3), the left-hand side is less than zero while the right-hand side is greater than zero. Therefore, each coefficient $a_i$ must be positive and non-zero.

2.1.2. Each non-zero coefficient $a_i$ must have the same magnitude as all the others

Let us assume that one or more of these $a_i$’s is greater than the others. We order the states in $n$, such that $a_2 \geq a_3 \geq a_4 \ldots \geq a_s$. Then $a_2, a_3, \ldots, a_m > a_{m+1}, \ldots, a_s$ (where $m$ is an integer between 2 and $r - 1$). If one of the states $m + 1, \ldots, r$ is connected to one of the states $2, \ldots, m$ (for definiteness and without loss of generality, let us say state 2 is connected to state $m + 1$), then $A_{2m+1}$ is a positive number. The left-hand side of Eq. (4) will be negative unless $a_2 < 1$. But, if that is true, then Eq. (3) cannot be satisfied. Thus, all $a_i$’s must be equal, and must all equal 1 to satisfy Eq. (3).

2.1.3. Having each coefficient equal to one is equivalent to having separate sub-networks

First, we note that the remaining rows $(r + 1$ through $s$) must sum to zero, since all of the rows together sum to zero. Next, looking at Eq. (3) with $a_2 = a_3 = \ldots = a_s = 1$, we see that the left-hand side (the coefficients $A_{ij}$ where $j > r$) must all be zero. The remaining $r$ equations all give the result that every coefficient $A_{ij} = 0$, where $i \leq r$ and $j > r$. We make the same argument for the coefficients $A_{ij} = 0$ where $i \leq r + 1$ and $j > r + 1$, since whatever holds for the first $r$ rows must hold for the remaining $s - r$ rows.

Therefore, we have shown that having each coefficient $a_i$ equal to one requires a matrix $A$, with two non-zero blocks along the greater diagonal and two zero-blocks along the lesser diagonal:

$$A = \begin{bmatrix} A_{ij} \mid 0 \\ 0 \mid A_{ij} \end{bmatrix} \quad (7)$$

This is equivalent to having two separate fully-connected networks, each governed by the non-zero blocks.

Thus, if a reaction matrix has two (or more) linearly independent vectors in its null-space, there must be two (or more) separate fully-connected sub-networks, and the reaction matrix must have non-zero sub-matrices along its diagonal and all other elements of the matrix must be zero. This concludes the proof.

2.2. Implications

Each fully connected reaction network has a single, unique steady-state solution, given that mass is conserved (i.e. a closed system). Therefore, in a reaction network made up of several fully-connected sub-networks, each sub-network must have a unique steady-state solution, given that mass is conserved within that sub-network. But, if mass is not conserved between sub-networks (in other words, there is a way to move mass from one sub-network to another), there may be numerous steady-state values. Thus, since muscle has many steady-state values for isometric force, there must be (at least) two separate fully-connected sub-networks, and the number of molecules in the subnetworks must be different for different isometric force values.

If we further make the assumption that it is the interaction of actin and myosin that leads to force enhancement, then for each steady-state where force enhancement occurs, there must be a different distribution of myosin cross-bridges among reaction sub-networks.

3. Force enhancement with cross-bridges

Before we apply this analysis of a general closed, first-order reaction network to cross-bridges, we must define certain aspects of acto-myosin interaction. Then, once these definitions are made, the previous analysis constrains the structure of cross-bridge reaction networks.

The following assumptions are made when defining a cross-bridge model:

1. Myosin exists in a finite number of discrete ‘states’.
2. Force comes from extension of a spring-like element in the myosin molecules.
3. Large numbers of cross-bridges are involved in contraction.
4. Actin and myosin cross-bridges interact with pseudo-first-order kinetics.
5. The binding of myosin to actin depends on the distance to the nearest actin binding site (from some reference frame on the myosin molecule) and on the state of myosin.
6. Sarcomeres are homogeneous.

These assumptions are by no means universally accepted. For example, there is evidence to suggest that sarcomere length homogeneity may explain some history-dependent contractile properties of muscle (see e.g. [32]); however, this evidence has been questioned (for a review, see Rassier and Herzog [41]). Some researchers have argued that cross-bridges do not act independently, but interact in some complex manner (e.g. [9, 2]). Depending on the nature of this interaction, the cross-bridges might not obey first order kinetics and/or myosin binding to actin may depend on other factors than distance from actin and myosin state.

Our purpose here is not to fully justify these assumptions; rather, it is to point out that these assumptions are commonly made in applications of such models. Thus, part of the goal of this paper is to investigate the effects of strain-dependence of the rate constants. We consider the stuck cross-bridge model. A stuck cross-bridge model is any model where there are some non-zero under others is to have that element be zero for some values of strain (x), and a second isometric distribution \( n_3^i(x) \) that then we have the following relation:

\[
F_{\text{iso}} = \sum_{U_i} \sum_{b_i} \int_{U_i} f_i(x)n_i^0(x) \, dx + \sum_{U_i} \sum_{b_i} \sum_{b_3} \int_{U_i} f_i(x)n_3^i(x) \, dx,
\]

where the functions \( f_i(x) \) relate displacement and force for a particular bound state, and we define \( n_i^0(x) \) to be the steady-state isometric cross-bridge distribution vector (in the subsequent equations, we drop the superscript s). Note that in the first term, we need not sum over \( S_i \) since there is only one fully-connected network in \( U_i \) by definition.

Force enhancement implies two different force values at steady-state for isometric contractions. Thus, if we have one steady-state isometric distribution \( n_i^0(x) \) and a second isometric distribution \( n_i^3(x) \), then we have the following relation:

\[
F_E = \sum_{U_i} \sum_{S_i} \sum_{b_i} \int_{U_i} f_i(x)(n_i^3(x) - n_i^0(x)) \, dx = 0. \quad (9)
\]

From this expression, we see that force enhancement depends only on the sub-domains that contain more than one sub-network. It is also clear that force enhancement depends only on the cross-bridges in sub-networks that contain at least some bound states.

We consider a special case of Eq. (9): all unbound states \((u_1, u_2, u_3, \ldots)\) are found in one subnetwork \( S_i \) for all \( U_i \). Then, all remaining subnetworks in each \( U_i \) contain only bound states. We refer to cross-bridge models of this type as ‘stuck cross-bridge’ models.

We consider another special case of Eq. (9): each subnetwork \( S_i \) contains at least one unbound state for all \( U_i \). Models of this type cannot be stuck cross-bridge models, since no subnetwork can contain only bound states. We refer to cross-bridge models of this type as ‘multi-cycle’ models.

The general form of Eq. (9) must be a mixture of the two models, since force enhancement requires a certain number of subnetworks that contain bound states – and those subnetworks either contain unbound states or they do not. If all of them lack unbound states they are stuck cross-bridge models; if all of them contain unbound states, they are multi-cycle models. Otherwise, they are a mixture of the two. An example of these two models is shown in Fig. 2. We will start by looking at the two models separately. First, we consider the stuck cross-bridge model.

3.1. Cross-bridge models that predict force enhancement

From the above analysis of reaction networks with two steady-state distributions, under isometric conditions there must exist two separate subnetworks in order to explain force enhancement. Also, under non-isometric conditions, there must be some way to move mass from one of these subnetworks to the other. Therefore, at least one element of the reaction matrix must be zero under isometric conditions and non-zero under non-isometric conditions.

Since the reaction network \( A(x) \) depends only on \( x \), the only way to have an element \( A_i \) be zero under some conditions and non-zero under others is to have that element be zero for some values of \( x \) and non-zero for others. Recall that each element \( A_i \) represents a (strain-dependent) rate constant. For a rate constant to be zero for some values of strain (x) and non-zero for others, a chemical reaction must become irreversible for some strains. We refer to such a reaction as a ‘strain-dependent irreversible reaction’. Therefore, given our assumptions of acto-myosin interaction and our assumption that acto-myosin interaction underlies force-enhancement, two different steady-state isometric force values requires at least one strain-dependent irreversible state transition.

In general then, we divide the domain \( x \) into several sub-domains with constant connectivity. We group these subdomains into regions with a single fully connected network (which we call \( U_1, U_2, U_3, \ldots \)) and regions with several separate networks (which we call \( U_1', U_2', U_3', \ldots \)). Each of these fully-connected networks and sub-networks we call \( S_i \), if it is the ith sub-network in the sub-domain \( U_i \) or \( U'_i \). Each of these networks is made up of a certain number of bound states \((b_1, b_2, b_3, \ldots)\) and a certain number of unbound states \((u_1, u_2, u_3, \ldots)\).

Then, we can write the force during an isometric contraction at steady-state as:

\[
F_{\text{iso}} = \sum_{U_i} \sum_{b_i} \int_{U_i} f_i(x)n_i^0(x) \, dx + \sum_{U_i} \sum_{b_3} \int_{U_i} f_i(x)n_3^i(x) \, dx,
\]

where the functions \( f_i(x) \) relate displacement and force for a particular bound state, and we define \( n_i^0(x) \) to be the steady-state isometric cross-bridge distribution vector (in the subsequent equations, we drop the superscript s). Note that in the first term, we need not sum over \( S_i \) since there is only one fully-connected network in \( U_i \) by definition.

Force enhancement implies two different force values at steady-state for isometric contractions. Thus, if we have one steady-state isometric distribution \( n_i^0(x) \) and a second isometric distribution \( n_i^3(x) \), then we have the following relation:

\[
F_E = \sum_{U_i} \sum_{S_i} \sum_{b_i} \int_{U_i} f_i(x)(n_i^3(x) - n_i^0(x)) \, dx = 0. \quad (9)
\]

From this expression, we see that force enhancement depends only on the sub-domains that contain more than one sub-network. It is also clear that force enhancement depends only on the cross-bridges in sub-networks that contain at least some bound states.

We consider a special case of Eq. (9): all unbound states \((u_1, u_2, u_3, \ldots)\) are found in one subnetwork \( S_i \) for all \( U_i \). Then, all remaining subnetworks in each \( U_i \) contain only bound states. We refer to cross-bridge models of this type as ‘stuck cross-bridge’ models.

We consider another special case of Eq. (9): each subnetwork \( S_i \) contains at least one unbound state for all \( U_i \). Models of this type cannot be stuck cross-bridge models, since no subnetwork can contain only bound states. We refer to cross-bridge models of this type as ‘multi-cycle’ models.

The general form of Eq. (9) must be a mixture of the two models, since force enhancement requires a certain number of subnetworks that contain bound states – and those subnetworks either contain unbound states or they do not. If all of them lack unbound states they are stuck cross-bridge models; if all of them contain unbound states, they are multi-cycle models. Otherwise, they are a mixture of the two. An example of these two models is shown in Fig. 2. We will start by looking at the two models separately. First, we consider the stuck cross-bridge model.

3.2. Stuck cross-bridge model

A stuck cross-bridge model is any model where there are some sub-domains of \( x (U_1', U_2', \ldots) \) that contain at least two unconnected subnetworks (e.g. \( S_1, S_2, \ldots \in U_i \)). One of these subnetworks, which we arbitrarily call \( S_i \), contains all of the unbound states. Note that bound cross-bridges in any subnetwork \( S_i \) in subdomain \( U_i \), where \( j \neq 1 \), cannot detach and are therefore ‘stuck’ on actin.

There is a unique distribution of cross-bridges in each \( S_i \), so for any two steady-state cross-bridge distributions \( n_i(x) \) and \( n_i(x) \) in that subnetwork we can write:

\[
k_{ii}^0(x) = \frac{n_i(x)}{n_i(x)}.
\]

Additionally, if the cross-bridge distributions are normalized, we may write:

\[
\sum_{n_i \in S_i} n_i(x) + \sum_{n_i \in S_i} n_i(x) = 1,
\]
where the first term is the fraction of cross-bridges cycling between bound and unbound states, and the second term is the fraction of cross-bridges that cannot unbind from actin, which we call \( N_{\text{stick}}(x) \). We can then rewrite Eq. (9) as:

\[
F_E = \sum_{\theta_i} \sum_{\eta_i \in S_i} \int f_i(x) \Delta n_i(x) \, dx + \sum_{\theta_i} \sum_{\eta_i \in S_i} \int f_i - \frac{\Delta N_{\text{stick}}(x)}{\sum_{\eta_i \in S_i} K_{\eta_i}(x)} \, dx. \tag{11}
\]

The first term in Eq. (11) is the change in force from the stuck cross-bridges (which is defined as \( \Delta F_i \)) due to their changing number and/or distribution. The second term is the change in force in the cycling cross-bridges (which is defined as \( \Delta F_c \)) due to changing the number of stuck cross-bridges. Thus, we obtain the expression:

\[
F_E = \sum_{\eta_i \in S_i} \Delta F_c(n_i(x)) + \sum_{\eta_i \in S_i} \Delta F_c(N_{\text{stick}}(x)), \tag{12}
\]

where \( n_i \) is a vector of the bound states in each subnetwork \( S_i \).

### 3.2.1. Velocity independence and length dependence

In order to match experimental measurements, force enhancement must depend on stretch magnitude while being approximately independent of stretch velocity (see Fig. 1). Here, we examine how these experimental observations constrain a stuck cross-bridge model. We start by considering a particular bound state \( \eta_i \). At some value of \( x \) at the border of subdomain \( U_i \), \( \chi_i \), the bound state becomes stuck to actin. In general, there may be several stuck states. Cross-bridges inside the stuck region may only move along with actin or switch between other stuck states. Since these cross-bridges cannot detach, they provide information about the state distribution before they entered the stuck region. In effect, each value of \( x \) in the stuck region provides a ‘snapshot’ of the cross-bridges just outside the stuck region some time \((x - \chi_i)/v)\) ago.

More formally, at some arbitrarily small distance \( e \) to the left of the stuck region, there is some time-dependent behavior for \( \eta_i(\chi_i^e - e, t) \). Given that \( \eta_i \) is continuous, we can write the following relation between the steady-state distribution of \( \eta_i \) in the stuck domain \((\eta_i^0) \) and the time-variant behavior of a point just outside the stuck region \((\eta_i(\chi_i^e - e, t)) \):

\[
\eta_i^0(x) = \sum_{b_i \in S_i} n_{\omega}(\chi_i^e - e, t \left( 1 - \frac{x - \chi_i}{\ell} \right)) \frac{K_{b_i}(x)}{\sum_{b_j \in S_j} K_{b_j}(x)},
\]

where \( v \) is the velocity of lengthening (assumed constant), \( \ell \) is the amount of lengthening and \( S_i \) is the particular subnetwork that contains \( \eta_i(x) \). We have implicitly assumed that \( k = 1 \), since we only wish to include stuck cross-bridges. Then, rewriting Eq. (11) we have, for stretch amplitudes larger than the largest stuck region:

\[
F_E = \sum_{\theta_i} \sum_{\eta_i \in S_i} \int_{\chi_i}^{\chi_i+L} h_{\eta_i}(x) \sum_{b_j \in S_j} n_{\omega}(x - e, t \left( 1 - \frac{x - \chi_i}{\ell} \right)) - g_{\eta_i}(x) \eta_i^0(x) \, dx, \tag{13}
\]

where \( h_{\eta_i}(x) \) is the initial isometric steady-state distribution, \( L \) is the length of \( U_i \), \( S_i \) is the subnetwork that contains \( b_j \) and \( g_{\eta_i}(x) \) and \( h_{\eta_i}(x) \) are defined as:

\[
g_{\eta_i}(x) = f_i(x) - \sum_{b_j \in S_j} f_j(x) \frac{K_{b_j}(x)}{\sum_{b_j \in S_j} K_{b_j}(x)}
\]

\[
h_{\eta_i}(x) = g_{\eta_i}(x) \frac{1}{\sum_{b_j \in S_j} K_{b_j}(x)}.
\]

For a given length \( \ell \), force enhancement is constant and independent of velocity. Therefore, \( dF_E/d\ell = G(\ell) \) and \( dF_E/dv = 0 \). Writing these expressions explicitly, we have:

\[
\frac{\partial F_E}{\partial \ell} = \sum_{\theta_i} \sum_{\eta_i \in S_i} \int_0^{\ell} h_{\eta_i}(x) - u \left( \sum_{b_j \in S_j} \frac{\partial n_{\omega}(x, t - u, t)}{\partial t} \right) \frac{u + t}{\nu} \, du = 0,
\]

where \( u = x - \chi_i \) and \( t = (u + t)/\nu \). And
\[ \frac{\partial F_k}{\partial t} = \sum_{U_i} \sum_{b \in S_i} \int_{0}^{t_1} h_j(x' - u) \left( \sum_{b_{u < S_i}} \frac{\partial n_{bw}(x' - \epsilon, t)}{\partial t} \right) \frac{1}{\nu} du = G(t). \]

The only way to satisfy Eqs. (14) and (15) is to pick rate constants and elasticity functions such that the following relation is true

\[ \left( \sum_{b_{u < S_i}} \frac{\partial n_{bw}(x' - \epsilon, t)}{\partial t} \right) h_j(x) \perp (u + \epsilon), \tag{16} \]

for all \( \epsilon \) and \( \nu \) over the sum of all of the domains \( U_i \). Note also that in order to satisfy Eq. (15), the system must not reach steady-state during stretch.

In general, then, it is possible to conceive of a stuck cross-bridge model that exhibits force enhancement with the correct stretch amplitude dependence and stretch velocity independence. A model would have a very special set of strain-dependent rate constants such that Eq. (16) is satisfied. Further, the system cannot reach steady-state during stretch, even for very long stretches.

3.3. Multi-cycle model

A multi-cycle model is any model where there are some sub-domains of \( X (U'_1, U'_2, \ldots) \) that contain at least two unconnected sub-networks (e.g., \( S'_1, S'_2, \ldots \in U'_i \)). Each of these subnetworks contain at least one unbound state. Therefore, in each subnetwork \( S'_i \) in subdomain \( U'_i \), cross-bridges cyclically bind and unbind to actin. In general, the properties of these cyclic interactions (such as the proportion of the cycle spent bound to actin, average force per cross-bridge, etc.) will be different for each subnetwork, and cross-bridges switch from one cycle to another only under particular values of strain (or equivalently, particular values of external load).

Combining Eq. (9) with Eq. (10), we find:

\[ F_E = \sum_{U_i} \sum_{b \in S_i} \int_{0}^{t_1} f_j(x) \frac{\partial n_{bw}(x' - \epsilon, t)}{\partial t} \sum_{n_{bw}} n_{bw}(x) dx. \]

Again, we see that force enhancement arises from moving cross-bridges between cycles.

Velocity-independence and the dependence on stretch magnitude do not place simple restrictions on this model. In the next section, when we consider a simple multi-cycle example, we will show that these constraints are naturally satisfied, so we delay discussion until then. One interesting prediction of this model is that very small amplitude stretches should show no force enhancement. Force enhancement in a multi-cycle model requires cross-bridges to be moved permanently from one cycle to another. This situation can only occur if some of the cross-bridges move into a region where two previously separated cycles interact, and then reenter a region where the cycles are once again separated. Thus, for small magnitude stretches, there can be no force enhancement.

4. Examples

At this point, we have established one main result of this paper: given the assumptions listed above, stuck cross-bridge models and multi-cycle models are the only way to explain force enhancement. However, up to this point, our analysis has been very general. In order to give an intuitive feel for the two classes of models, we provide examples of each. The purpose of this section is to reinforce the previous analysis with examples, and to provide an intuition for later arguments (e.g., that it is unlikely for a stuck cross-bridge model to have the correct velocity and stretch amplitude dependence while these relations are natural for a multi-cycle model).

Perhaps the conceptually simplest cross-bridge model for muscle contraction is the Huxley 1957 model, modified as in [50] to fit lengthening steady-state force. This model has an analytic solution for steady-state, and it is relatively easy to solve the full equations numerically (compared to models with more realistic kinetics). So, by modifying this model slightly we will examine a stuck cross-bridge model and a multi-cycle model. We do not intend these to be the correct model for muscle, rather we show the qualitative behavior of such a model.

4.1. Stuck cross-bridge example

The Huxley rate functions \( f(x) \) and \( g(x) \) can be modified to create a stuck cross-bridge model by setting \( g(x) \) to zero for some region. This model produces force enhancement if the stuck region occurs at large strains where the attachment function \( f(x) \) is zero, as in Fig. 2. We can estimate the force enhancement from a quasi-steady-state solution to Huxley’s equations (see Appendix A for derivation):

\[ F_E = \begin{cases} A(1 - e^{-\frac{L_s}{L}}) e^{-\frac{L_s}{L} + \frac{x}{L}} : & \epsilon \leq L_s, \\ A(1 - e^{-\frac{L_s}{L}}) e^{-\frac{L_s}{L} + \frac{x}{L} - \frac{L_s}{L}} : & \epsilon > L_s, \end{cases} \tag{17} \]

where \( A, B \) and \( C \) are constants dependent on the parameters of the Huxley model, \( L_s \) is the length of the ‘stuck’ cross-bridge region, \( h \) is defined in Fig. 2, and \( \epsilon \) is the stretch magnitude.

With an appropriate choice for \( A, B \) and \( C \), it is possible to achieve velocity insensitivity, while absolute velocity independence for force enhancement is impossible with this modified Huxley model – thus demonstrating the difficulty in satisfying Eq. (16). It can be seen in Fig. 3 and from Eq. (17) that force enhancement ceases for stretches larger than \( L_s \). If we define the spacing between adjacent actin binding sites as \( 2L \), this region is bound by \( L_s < 2L - h \). Thus, in this model, we cannot see any length dependence of stretch for stretches larger than \( 2L - h \). In other words, the ‘memory’ of this model is erased for large stretches.

4.2. Multi-cycle example

A multi-cycle model can be created by coupling two Huxley-type models with different isometric force values (that is, different slopes for \( f(x) \) and \( g(x) \)). The bound state of the first cycle is connected to the unbound state of the second cycle by a function \( (\omega(x), h \omega(x)) \), which is only non-zero for strains larger than \( h \) (see Fig. 2). If we assume that steady-state within cycles is reached quickly (a quasi-steady-state approximation), we may find an analytic solution for the cross-bridge attachment distributions, the time-dependent force, and force enhancement (see Appendix B). The force enhancement equation is of the form:

\[ F_E = \begin{cases} 0, & n = 0, \\ \Delta F_{iso} N_0 (1 - (1 - a)^{-n}) : & f \leq 1/2, n > 0, \\ \Delta F_{iso} N_0 (1 - (1 - a)^{-n}) \left(1 - a \left(\frac{L_s}{L_s - n} \right)^2 \right) : & \frac{hb}{L_s - n} \leq f < \frac{1}{2}, n > 0, \\ \Delta F_{iso} N_0 (1 - (1 - a)^{-n}) : & f > \frac{1}{2}, n > 0, \end{cases} \tag{18} \]

where \( a(v) \) is a positive velocity-dependent function less than 1, \( \Delta F_{iso} \) is the difference in isometric force between cycles 2 and 1, \( N_0 \) is the initial (assumed uniform with \( x \)) proportion of cross-bridges in cycle 1, \( n \) is the integer part of \( \epsilon/2L \) and \( f \) is the fractional part of \( \epsilon/2L \).

From Eq. (18), we see that force enhancement requires a stretch of at least \( 2L \), and has an asymptote at \( F_{iso} \). If \( a(v) \) is relatively constant, then force enhancement will have little velocity dependence, while maintaining a strong length dependence (see Fig. 4). For a
A wide range of choices for the parameters describing the \( f(x) \) and \( g(x) \) functions, \( a, v \) has very weak velocity dependence – indicating that the velocity independence of force enhancement is not a very stringent constraint on the multi-cycle model.

The quasi-steady-state time-dependent force can be obtained analytically for an isokinetic stretch (see Appendix B). This equation predicts a slow (step-like) increase in force for prolonged stretches (see Fig. 5). This slow rise in force is observed in most isokinetic stretches (as noted by Edman et al. [11]).

The numerical solution of the full equations for this model agrees well with the quasi-steady-state approximation, though the rapid response to stretch initiation and slow decay towards the final force after stretch are not replicated (see Fig. 5). Thus, a simple Huxley-type, two-cycle model can reproduce force

Fig. 3. Velocity and stretch amplitude dependence for the simplest stuck cross-bridge model. (A) Steady-state force as a function of velocity (solid) and isometric force after stretch using the quasi-steady approximation (dotted) – the stretch magnitude (in sarcomere lengths) labels each curve. The velocity dependence matches that seen in experiments (see e.g. [11]). Inset: the force–velocity curve for this model. This curve is included to show that the parameters were chosen in such a way that a qualitatively correct force–velocity curve is generated by the model. (B) Force enhancement using the quasi-steady approximation (as percent of isometric force) as a function of stretch amplitude (in sarcomere lengths). Each curve is labeled with the velocity of stretch. The curves level off at very small stretch magnitudes. This is not consistent with experiments.

Fig. 4. Velocity and stretch amplitude dependence for the multi-cycle cross-bridge model. (A) Steady-state force as a function of velocity (solid) and isometric force after stretch using the quasi-steady approximation (dotted) – the stretch magnitude (in sarcomere lengths) labels each curve. The velocity dependence matches that seen in experiments (see e.g. [11]). Inset: the force–velocity curve for this model (for the first cycle). This curve is included to show that the parameters were chosen in such a way that a qualitatively correct force–velocity curve is generated by the model. (B) Force enhancement using the quasi-steady approximation (as percent of isometric force) as a function of stretch amplitude (in sarcomere lengths). Each curve is labeled with the velocity of stretch. The curves are consistent with those found in experiments (see e.g. [12]).
enhancement, with appropriate velocity independence, stretch amplitude dependence and (somewhat unexpectedly) can also reproduce the slow, long lasting force rise seen during stretch.

5. Why the stuck cross-bridge model is unlikely

It might seem that the stuck cross-bridge model is the most likely explanation for force enhancement. It has been shown that applied force changes the kinetics of ADP release in smooth muscle [49,25]. In addition to ADP release, the power stroke might be load-dependent. It therefore seems possible that very slowly detaching cross-bridges might be the cause of force-enhancement. The resulting kinetic scheme would be a stuck cross-bridge model.

However, in the simplest stuck cross-bridge model example, force-enhancement is only dependent on stretch amplitude for very small stretches (see Fig. 3), in contrast to experimental measurements (see Fig. 1). Here we explain this result. From this explanation and some known properties of myosin, we argue that the stuck cross-bridge model is an unlikely explanation for force enhancement in muscle.

5.1. A single detached state

Here, we prove that any cross-bridge model with a single detached state may only have stretch amplitude dependence for stretches less than or equal to 2L. We then show some cases where this result holds for an arbitrary number of detached states.

We start with the observation that there exists at least one value of x, x = −L, where no cross-bridges may bind. This observation follows from the assumption that binding to actin depends only on the distance to the nearest binding site (assumption 5 in Section 3).

If myosin is equidistant from two binding sites, then neither is closer and the cross-bridge may not bind.

5.1.1. An ensemble of cross-bridges with a single detached state starting from x = −L will have a periodic η(ζ, t), with period T = 2L/ν

We define \( t_j = t - 2L/ν \). If we choose \( j \) to be the integer part of \( νt/2L \), then \( t_j \) is the time since the most recent moment that \( x = −L \). Note that \( x = νt_j = L \).

We define \( η(t) = η(ζ = −L, t) \). Let us assume that the detached state is the first entry of \( η_j \). Every time that \( t_j = 0, x = −L \) by definition. From the previous observation that cross-bridges may not bind at \( x = −L \), for any integer \( j, η_j(t_j = 0) = 1 \) and for all \( i \neq 1 \) \( η_i(t_j = 0) = 0 \).

The time evolution of \( η \) is governed by Eq. (8), repeated in modified form below:

\[
\frac{dη}{dt_j} = A(νt_j - L)η(t)
\]

The solution to this equation, \( η(t) \), is uniquely determined given an initial condition \( η(0) \). Therefore, since \( η(t_j = 0) \) is a constant for all \( j \), and since \( t_j \) is periodic with period \( T = 2L/ν \), \( η(t) \) must also be periodic with period \( T = 2L/ν \).

5.1.2. Any ensemble of cross-bridges with arbitrary \( i \) will have periodic \( η_i(ζ, t) \), with period \( T = 2L/ν \), after at most \( t = 2L/ν \)

After a stretch of \( (L - ζ), x = −L \). At this point, by the argument of the previous section, \( η_i(ζ, t) \) will be periodic with period \( T = 2L/ν \). More specifically, if we call this time \( t^* \), then we may write:

\[
η\left(t - \frac{L - ζ}{ν}\right) = η_i(ζ, t) \quad t \geq t^*.
\]
Since, \(-L < x < L\) when \(t = 0\), the largest stretch at which \(x = -L\) is less than \(2L\). Thus \(t' < 2L/\nu\).

5.1.3. The proof

We now prove that for a system with a single unbound state, force-enhancement is constant for stretches of amplitude \(2L\) or greater.

The exact definition of \(\xi\) for a constant speed stretch is \(\xi = x - \text{mod}(vt, 2L)\). However, since \(\eta(t + j2L/\nu) = \eta(t)\), where \(j\) is an arbitrary integer, we may substitute \(\xi = x - vt\) in Eq. (19). Thus,

\[
\mathbf{n}(x, t) = \eta \left( \frac{x - L}{\nu} \right), \quad t \geq t'.
\]

(20)

Clearly, \(\mathbf{n}(x, t)\) has no time dependence, and thus all of its properties are time-independent. Therefore, force-enhancement must become constant for \(t \geq t'\). We know that \(t = t'\) at a stretch amplitude of \(2L\), so therefore force-enhancement is constant for stretches of amplitude \(2L\) or greater.

Comment 1: At no point in this proof did we assume anything about the matrix of rate constants \(A(x)\), nor about the length of the vector \(\mathbf{n}(x, t)\), nor about the number of subnetworks or their connectivity. We simply assumed that there was a single unbound state.

Comment 2: The above proof depends on there being one value of \(x\) where the cross-bridge distribution is time-invariant. In a sense, the 'memory' of the system is erased as cross-bridges pass through these regions. In the next section, we consider a system with two or more detached states.

5.2. Multiple unbound states

For most physically based cross-bridge models, there is a range over which cross-bridges are bound with negligible probability. Let us call this range \(L_0\). While in this region, the unbound cross-bridges can potentially interchange between other unbound states. In a stuck cross-bridge model, by definition, these unbound states form a fully-connected network. While it takes an infinite time to reach steady-state, these unbound states may come very close to steady-state in finite time.

If these unbound states come sufficiently close to steady-state in the time \(L_0/\nu\), then force-enhancement will be essentially constant for stretches of amplitude \(2L\) or greater. In particular, we claim that if the unbound states differ from steady-state by a vector whose positive entries sum to \(\delta\), then at any time \(|n_i(t, t_{\text{ub}} + t) - n^{0}_i(x)| \leq \delta\) where \(t_{\text{ub}} = 2L/\nu\) and \(n^{0}_i(x)\) is the steady-state distribution. Consequently, for stretches greater than \(2L\):

\[
\Delta F_E \leq \delta \sum_{\{i\}} \sum_{j < i} \sum_{k < j} \int_{U_{ij}} |f_j(x)| dx.
\]

5.2.1. Proof

Since the governing differential equation for \(n_i(\xi, t)\) is linear, there exists some linear operator \(T(t')\) such that \(n_i(\xi, t + t') = T(t') n_i(\xi, t)\). We assume that there exists a range where cross-bridges do not bind to myosin. After passing through this range (which we assume happens at \(t = t'(\xi)\)), the distribution of unbound states may be written \(n_{\text{ub}} = n_0 + \mathbf{d}\), where the sum of the positive entries of \(\mathbf{d}\) is \(\delta\) (note that since the sum of the entries of \(\mathbf{d}\) is zero, \(\delta = 1/2\sum_{i} |d_i|\)).

By the linearity of the operator \(T(t')\),

\[
\mathbf{n}(\xi, t' + t) = T(t') \mathbf{n}_0(\xi) + \delta T(t') \mathbf{d}.
\]

There exist two normalized distributions \(\mathbf{n}_1\) and \(\mathbf{n}_2\) such that \(d_i/\delta = n_1(i, t) - n_2(i, t)\). Thus,

\[
T(t') \mathbf{d}/\delta = T(t') \mathbf{n}_1(\xi, t) - T(t') \mathbf{n}_2(\xi, t).
\]

(21)

Each entry of the vectors on the right-hand side of Eq. (21) is between 0 and 1. Consequently, each entry of \(T(t') \mathbf{d}/\delta\) can have a maximum magnitude of 1. Therefore, using the fact that \(T(t') \mathbf{n}^{0}_i(\xi, t'(\xi)) = n^{0}_i(x)\), we have

\[
|n_i(\xi, t'(\xi) + t') - n^{0}_i(x)| \leq \delta.
\]

(22)

Note that Eq. (22) will hold if we replace \(t'(\xi)\) with the upper bound on \(t'\), which is \(t_{\text{ub}} = 2L/\nu\). Then Eq. (22) holds for all \(\xi\), and consequently

\[
|n_i(x, t_{\text{ub}} + t') - n^{0}_i(x)| \leq \delta.
\]

(23)

Since force enhancement increases monotonically with stretch magnitude, it follows that maximal force enhancement occurs when steady-state is reached during stretch. Consequently, we may use Eq. (23) to put an upper bound on force enhancement for \(t > t_{\text{ub}}\) (using Eq. (9)):}

\[
\Delta F_E \leq \delta \sum_{\{i\}} \sum_{j < i} \sum_{k < j} \int_{U_{ij}} |f_j(x)| dx.
\]

5.3. Parameter estimation

We may estimate an upper bound to force enhancement from experimental measurements.

Consider a stretch of 0.1 sarcomere lengths per second for a 2.5 μm sarcomere, \(v = 130 \text{ nm/s}\). Assuming a stiffness of 0.7 pN/nm [48, 34, 31] and assuming a 10 nm power-stroke [15], a cross-bridge strain of 18 nm would predict a load of about 20 pN on a cross-bridge during lengthening. Estimates for the maximum force needed to break a rigor bond, the upper bound on the force a cross-bridge may hold, vary from 10 to 15 pN [33–35]. Thus, it seems likely that most cross-bridges will be unbound before achieving 18 nm of strain. In order to achieve negative strain values during lengthening, a detached cross-bridge must be excited by thermal agitation and bind to actin. With a cross-bridge stiffness of 0.7 pN/nm, an unloaded binding rate of 20 s⁻¹ [29] and a lengthening rate of 130 nm/s, the probability that a detached cross-bridge achieves a strain of −10 nm is 0.0015% at 30°C. Therefore, since \(2L = 36\) nm, we may estimate the size of the region where cross-bridges may not bind: \(L_0 \approx 8\) nm.

There are typically thought to be two major unbound states, myosin with ATP bound (MT), and myosin with hydrolysis products bound (MDP). Using estimates of the rate constants between the unbound states from Pate and Cooke [37] (ATP hydrolysis at 100−1 s⁻¹ and formation at 10 s⁻¹), we find that even when the unbound states are as far from equilibrium as possible (i.e. completely in the MT state), by the end of \(L_0\) each of the two states is within 0.00096 of its equilibrium value. Therefore, we may set an upper bound \(\delta < 0.00096\).

Typically, it is thought that there are three strongly bound states: a pre-power-stroke state, a post-power-stroke state and a rigor state (e.g. [3]). We assume that force comes from extension of a linear spring, \(f_j(x) = k(x + x^0_j)\), where \(x^0_j\) is the displacement associated with each state. For the pre-power-stroke state, \(x_0 = 0\). For the remaining two states \(x_0 < 10\) nm [15]. Then,

\[
\Delta F_E = \frac{\delta}{2T} \left( \int_{-10}^{18} kx dx + \int_{-10}^{18} k(x + 10) dx \right),
\]

where we integrate over the whole range that cross-bridges may bind.

Plugging in \(k = 0.7\) pN/nm and \(\delta = 0.00096\), we have:

\[
\Delta F_E < 0.0186\text{ pN}.
\]
Given an isometric force per head of 0.75 pN [7] (which is likely an underestimate because there is evidence that only one head of myosin interacts with actin at a given time [24]), this corresponds to \( \Delta F \leq 2.5\% \).

Therefore, after an initial stretch of 36 nm, force enhancement could further increase by less than 2.5\% for arbitrarily large stretch amplitudes. Note that our parameter estimates were purposefully conservative, so that this number represents an upper bound. From Fig. 1, we see that the data show that force enhancement increases more than this upper bound. Therefore, we conclude that the stuck cross-bridge model is very unlikely.

Thus, it seems unlikely that a stuck cross-bridge model can explain force-enhancement. Additionally, a stuck cross-bridge model must still satisfy Eq. (16). This relationship implies that the velocity-dependent time history of the binding probability of cross-bridges just outside the stuck regions must have special properties for all velocities. There is no obvious reason why this relationship should be satisfied. On the other hand, in the multi-cycle model, these restrictions are much more naturally satisfied. We conclude that multi-cycle models are a more likely explanation for force enhancement.

6. Discussion

It has been proposed that there are five major acto-myosin–ATP complexes, two with actin and myosin unbound and three bound. In the absence of strain, it appears that each of these complexes is associated with a particular configuration of the myosin S1 domain [43]. In the presence of strain, there could be many different myosin and actin configurations within this five-complex framework, and so by the definition used here) there may be many more than five states. For example, it has been suggested that strain on a bound cross-bridge state (the myosin–actin–ADP or strongly bound ‘state’) may decrease the rate of cross-bridge detachment [36,44]. In smooth muscle myosin (which has different cross-bridge kinetics from skeletal muscle), there is evidence that cross-bridges can become stuck on actin (the so-called ‘latch state’ [43,44]). This strain-induced conformational change in a molecule. For such a reaction, the free energy barrier is too large to occur under physiological temperatures.

If we accept that force enhancement occurs through a multi-cycle cross-bridge model, it remains to be explained how this force enhancement disappears upon deactivation. One possible explanation is that the troponin–tropomyosin complex, in the absence of calcium, interacts with myosin catalyzing the state transition between the myosin configurations such that it can occur under physiological conditions, perhaps in a similar fashion as a chaperone (see Fig. 6). The slow disappearance of these force-enhanced cross-bridges may be related to the ‘passive’ force enhancement after deactivation.

These explanations are speculation and have not been rigorously tested experimentally. Their purpose is to show that plausible mechanisms for the multi-cycle model exist, so that we cannot dismiss it a priori, and thus we cannot discount cross-bridge interaction as the mechanism of force enhancement following stretch. If specific evidence is found to support the multi-cycle model, then these questions may become useful to the understanding of muscle function.

Recent investigations of the load dependence of rate constants in a single smooth muscle myosin molecule [49,25], suggest a method to test the multi-cycle model directly with the three bead assay in the laser trap. Application of a transient load to skeletal muscle myosin during attachment could pull the cross-bridge through the strain-dependent state transition into a different kinetic cycle. If multiple cycles are present, the duration of attachment may be significantly altered following load application. Rapid event detection and load application techniques (e.g. [47]) may enable us to investigate all of the bound states, even those with short lifetimes. If after careful investigation, it appears that the multi-cycle model is not correct, then it can be concluded that force-enhancement lies outside acto-myosin interaction, or must depend on significant cross-bridge interactions even at maximal activation. If evidence is found for the multi-cycle model, it would represent a discovery not only for muscle, but for the study of en-
zymes. The possibility of strain-dependent 'memory' in an enzyme is very exciting, and would represent a new and surprising biochemical mechanism.

The analysis presented above depends on the six assumptions listed in Section 3. Though most of these assumptions are quite common in mathematical muscle models, there is evidence to suggest that some of them are not correct. For example, assumption 5, that cross-bridge kinetics depend only on the distance to the nearest actin binding site, rules out cooperative binding [40]. The effect of violating these assumptions on cross-bridge force generation, and in particular on force-enhancement, is not completely clear. Here, we have shown that force enhancement may be explained without violating these assumptions. This, in itself, is an important finding. Further, we have shown that such a cross-bridge model would require multiple cycles and a long-lasting load-dependent conformational change. We may therefore examine the mechanism of force enhancement by looking for such a reaction with the experiments described above. If evidence of this reaction cannot be found, then we may add weight to the argument that alternate mechanisms, such as cooperativity or calcium-dependent stiffness of 'passive' elastic proteins (e.g. titin), are responsible for the currently unexplained phenomenon of force enhancement. Additionally, we could conclude that force enhancement results from violating our assumptions and could focus our efforts on trying to understand these effects. Therefore, this work remains valid although some of our assumptions might be questionable.

It may seem that the preceding analysis depends on the assumption that two steady-state force values exist at a given length. The experimental evidence cannot rule out the possibility that force enhancement disappears after a sufficiently large time (e.g. much longer than 30 s). Transient, but long-lasting, force enhancement does not change the result of this work. Long-lasting force increase requires a strain-dependent slowly reversible state transition. Only two models exist with this type of state transition. In the first model, strained cross-bridges in a particular state are strongly attached to actin (though not indefinitely stuck). In the second model, two cycles are connected by a strain-dependent slowly reversible state transition. The second model, essentially the multi-cycle model described earlier, is the only model that can reproduce the stretch amplitude dependence of force enhancement. The result of our analysis is thus still accurate if force enhancement is long-lasting rather than indefinite.

7. Conclusions

With new technologies for looking at the interaction of actin and myosin molecules, it is of great interest to understand how these molecules interact in working muscle. The wealth of data collected over the past hundred years provides an opportunity to propose physically based models aimed at teasing out the coupling between macroscopic force and molecular interactions. Here, we have shown that it is possible to explain the history dependence of muscle force with the interaction of individual myosin molecules with actin. We also show that the only way to do so is with a multi-cycle model that involves a strain-dependent irreversible state transition, a claim that can be tested. If evidence can be found for such a state transition, then the lessons learned from experiments on single molecules can be readily generalized to the level of myofilaments, muscle fibers and even whole muscle. However, if evidence cannot be found for such a state transition, then either cooperativity (or other inter-myosin interactions), other molecules or sarcomere (or other structural) non-uniformity must contribute significantly to macroscopic muscle force. In that case, the generalization of single molecule experiments becomes more difficult.

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Appendix A

The following is a quasi-steady-state solution to a Huxley-type stuck cross-bridge model as shown in Fig. 2. The attachment function \( f(x) \) is defined as

\[
0: \quad x < 0,
\]

\[
f(x) = \begin{cases} 
0: \quad x > h, 
\end{cases}
\]

and the detachment function \( g(x) \) is defined as

\[
g(x) = \begin{cases} 
g_0: \quad x < 0, 
g_1: \quad h \geq x \geq 0, 
g_2: \quad x \geq h, 
g_3: \quad x > x_c + L_c - h, 
g_4: \quad x > x_c + L_c, 
g_5: \quad x_c > h, 
g_6: \quad x > x_c + L, 
g_7: \quad x > x_c + L_c. 
\end{cases}
\]

The steady-state distribution of attached cross-bridges \( \nu_0^a(x) \) is the solution to the following differential equation

\[
u \frac{d\nu_0^a(x)}{dx} = f(x)(1 - \nu_0^a(x)) - g(x)\nu_0^a(x).
\]

Solving this differential equation for muscle undergoing stretch \( \nu > 0 \):

\[
\nu_0^a(x) = \begin{cases} 
0: \quad x < 0, 
\frac{k}{\nu} \left(1 - \exp\left(-\frac{g_1 - g_2}{\nu}\right)\right): \quad h \geq x \geq 0, 
\end{cases}
\]

\[
\nu_0^a(x) = \begin{cases} 
\nu_0^a(h) \exp\left(-\frac{(x-h)(g_1(x-h)+2bg_1)}{2\nu}\right): \quad x_c \geq h, 
\nu_0^a(x_c): \quad x_c + L_c \geq x \geq x_c, 
\nu_0^a(x_c): \quad x > x_c + L_c, 
\end{cases}
\]

To estimate the force and the state distributions as a function of time, we make a quasi-steady-state assumption. If we start from an isometric distribution, then this assumption means that the steady-state distribution propagates at the speed \( \nu \). To further simplify the equations we assume that the steady-state distribution replaces the isometric distribution in the region \( x < x_c \) after a delay of \( t_p = (x_c - h)/\nu \). In our simulations, we found that most of the transient effects died out by \( t_p \). We define the propagation length \( \ell = \nu t_p + h \). Then, the quasi-steady attached cross-bridge distribution \( \nu_0^a(x,t) \) is:

\[
\nu_0^a(x,t) = \begin{cases} 
0: \quad x < 0, 
\frac{k}{\nu} \left(1 - \exp\left(-\frac{(x-x_c+L)(g_1(x-x_c+L)+2bg_1)}{2\nu}\right)\right): \quad h \geq x \geq 0, 
\end{cases}
\]

\[
\nu_0^a(x,t) = \begin{cases} 
\nu_0^a(h)H(\ell - x_c): \quad x_c \geq h, 
\nu_0^a(x_c)H(\ell - x_c): \quad x_c + L_c \geq x \geq x_c, 
\nu_0^a(x_c)H(\ell - x_c): \quad x > x_c + L_c, 
\end{cases}
\]

where \( H(y) \) is the Heaviside function which is zero for \( y < 0 \) and one for \( y \geq 0 \). Thus, we can estimate the force as:
where $\kappa$ is the spring constant of the (assumed linear) springs on the cross-bridges. Eq. (24) becomes:

$$F(t) \approx \frac{1}{2L} \int_{0}^{L} \kappa \pi r_{0}^{2}(x,t) \, dx,$$

where $\kappa$ is the spring constant of the (assumed linear) springs on the cross-bridges. Eq. (24) becomes:

$$F(t) \approx F_{iso} + (F_{s} - F_{iso})H(\ell - x_{c}) + \cdots + \frac{n_{0}^{2}(x_{c},t,\kappa)}{2L} \left( \ell^2 + ((x_{c} + L_{s})^2 - \ell^2)H(\ell - x_{c} - L_{s}) - x_{c}^2 \right) + \cdots$$

$$\times \int_{x_{c}+L_{s}}^{\ell} \exp \left( \frac{-(x-x_{c}-L_{s})}{2} \right) \left( g_{s}(x+x_{c}+L_{s}-2h) + 2h g_{s} \right) \, dx.$$

(25)

Here, $F_{s}$ is the steady-state force that would be measured for a force–velocity plot (i.e. the force predicted by a ‘traditional’ Huxley model with $L_{s} = 0$). The integral in Eq. (25) can be evaluated analytically, but its solution is quite long. Thus, the force response occurs in three phases: the first is the rapid transition to stretch. After a short transition time, the quasi-steady approximation is good. The shaded region indicates the time of muscle stretch. After a short transition time, the quasi-steady approximation is good. (B) Stretch amplitude dependence of force enhancement at three different stretch velocities. Each dot is the result of a numerical simulation of the full simplest stuck cross-bridge model. The dotted lines are the quasi-steady approximations. The approximations are good, especially at slower stretch velocities.

Note that $F_{s}$ is zero for $\ell < x_{c}$ and constant for $\ell \geq x_{c} + L_{s}$. Thus, force enhancement in this model can only have a length dependence for stretches on the order of the stuck region $L_{s}$.

**Appendix B**

The following is a quasi-steady-state solution to a Huxley-type multi-cycle model. In this model, there are two separate traditional Huxley-type models, where upon strain, the attached state of one model can transition to the detached state of the other model with a rate function $w(x)$. There are two attachment functions, $f^{1}(x)$ and $f^{2}(x)$, defined as follows:

$$f^{i}(x) = \begin{cases} 0 : & x < 0, \\ f^{i}(x) : & h \geq x > 0, \\ 0 : & x > h, \end{cases}$$

where $i = 1$ or 2. There are also two detachment functions, $g^{1}(x)$ and $g^{2}(x)$, defined as follows:

$$g^{i}(x) = \begin{cases} g^{i}_{0} : & x < 0, \\ g^{i}_{1} : & h \geq x > 0, \\ g^{i}_{1}h + g^{i}_{2}(x-h) : & x > h. \end{cases}$$

And, the transition function $w(x)$ is defined as:

$$w(x) = \begin{cases} 0 : & x < h, \\ w_{1}(x-h) : & x > h. \end{cases}$$

There are two attached states, whose steady-state distributions $n_{0}^{1}(x,v)$ and $n_{0}^{2}(x,v)$ satisfy the following differential equations:
\[
\begin{align*}
\frac{dn_n^1(x)}{dx} &= f^1(x)(N_1(x) - n_n^0(x)) - (g^1(x) + w(x))n_n^0(x), \\
\frac{dn_n^2(x)}{dx} &= f^2(x)(1 - N_1(x) - n_n^0(x)) - g^2(x)N_n^2(x),
\end{align*}
\]

where \(N_n(x)\) is the proportion of cross-bridges in the first cycle. In this model, the first part of the quasi-steady-state approximation is that these differential equations are satisfied for all time and that all time dependence occurs in \(N_1(x, t)\).

Here we derive an expression for the time-dependence of \(N_1(x, t)\). Cross-bridges leave cycle 1 at large strains at a rate \(w(x)\). We assume that at time \(t = 0\), the proportion of cross-bridges in the first cycle is independent of \(x\) (\(N_1(x, 0) = N_0\)). As lengthening begins, cross-bridges move from cycle 1 to cycle 2. The quasi-steady-state assumption implies that the differential equation for the steady-state value of \(N_n^1(x)\) is satisfied:

\[
\frac{dN_n^1(x)}{dx} = -w(x)n_n^0(x). \quad (27)
\]

From the form of \(w(x)\), it is clear that \(dN_1(x)/dx\) is zero except during the interval \(h \leq x \leq L\). Furthermore, we know that \(n_n^0\) must approach zero as \(x\) approaches \(L\) (because of the assumption that cross-bridges interact only with the nearest binding site). Therefore, the solution of Eq. (27) must be some constant value for \(-L < x < L\) and some function for \(h < x < L\) that asymptotes to a potentially different constant at \(x = L\). In any Huxley-type model, periodic boundary conditions are assumed (see e.g. [37,14,23]). Thus, the fact that our solution of Eq. (27) at \(x = L\) is potentially different from the solution at \(x = L\) violates this boundary condition. One way around this potential contradiction is to allow a discontinuity in \(N_n^1(x)\). Therefore, the second part of our quasi-steady-state approximation is that \(N_n(x, t)\) is the solution to \(N_n^1(x)\) with a time-dependent discontinuity.

For an initially isometric cross-bridge distribution, this discontinuity begins at \(x = h\). As these attached cross-bridges are stretched, some of them switch from cycle 1 to cycle 2 (and some detach). If we assume that the steady-state distribution is reached instantaneously, then this discontinuity propagates at speed \(v\). So, at any time \(t\), we find the discontinuity at \(x = (h + vt + L)/\text{mod}(2L) - L\). Or, if we define the variable \(n\) to be the integer part of \((h + vt + L)/2L\), and the variable \(f\) to be the fractional part of \((h + vt + L)/2L\), we find the discontinuity at \(x = 2Lf - L\).

Let us define a family of solutions to Eq. (27). The first, \(N_1^1(x, t)\), starts at \(N_0\) with \(x = -L\) and then remains constant in \(x\) until \(x = h\). It then asymptotes to a new value at \(x = L\). Each subsequent solution, \(N_t^1(x, t)\), starts at \(N_n^1(L)\), and remains constant until \(x = h\).

\[
N_n^1(x, t) = \begin{cases} 
0 : & (f_n^1(x,t) + g_n^1(x,t) + w_n) \left( 1 - \exp \left( \frac{-(x-h)(x+h)}{2v} \right) \right) \\
1 : & (f_n^1(x,t) + g_n^1(x,t) + w_n) \left( 1 - \exp \left( \frac{-(x-h)(x+h)}{2v} \right) \right) \exp \left( \frac{-(x-h)(x+h)+w_n(x-h)+2g_n^1(x)}{2v} \right) 
\end{cases}
\]

at which point it asymptotes to a new value at \(x = L\). To this family of solutions, we append the initial distribution \(N_n^0(x) = N_0\). The quasi-steady approximation of \(N_1(x, t)\) is then \(N_n^1(x)\) for \(x = -L\) until the discontinuity at \(x = 2Lf - L\) above which it is \(N_n^0\). Thus, to find \(N_1(x, t)\), we need only find \(N_n^1(x)\) (see Fig. 8).

We may further simplify the problem by noting that the attached distributions \(n_n^1\) and \(n_n^2\) depend on the value of \(N_n(x, t)\) only over the interval \(0 \leq x \leq h\). Thus, it is not necessary to know the exact form of \(N_n(x)\) for \(h < x \leq L\), we need only know the limit

\[
\begin{align*}
F(t) &\approx \frac{1}{2L} \int_{-L}^{L} k(x(n_n^1 + n_n^2)) \, dx.
\end{align*}
\]
We solve this equation for \( n > 0 \), and then show how \( n = 0 \) can be added. We start by defining the following functions:

\[
B'(x) = \frac{f_1}{f_1 + g_1} \left( 1 - \exp \left( -\frac{(f_1 + g_1)x}{2}\right) \right),
\]

\[
C'(x) = B'(x) \exp \left( \frac{-h(x)}{2} \right) \left( \frac{f_1 + 2g_1 h(x)}{f_1 + g_1} \right).
\]

where \( j = 1, 2 \) and \( w_0 = 0 \). Thus, \( B'(x) \) is the steady-state force that would occur if all of the cross-bridges were in cycle \( j \). Note that \( B'(x) \) and \( C(x) \) are always positive, as are \( \kappa \) and \( \chi \) (over the range of integration). Then, we may write \( F(t) \) as:

\[
F(t) = \begin{cases} 
\int_{-\infty}^{f_1} \frac{2\kappa}{f_1 + g_1} \kappa B(x) \, dx + \int_{f_1}^{\infty} \frac{2\kappa}{f_1 + g_1} \kappa C(x) \, dx, & \text{if } f < 1/2, \\
\int_{-\infty}^{f_1} \frac{2\kappa}{f_1 + g_1} \kappa B(x) \, dx + \int_{f_1}^{\infty} \frac{2\kappa}{f_1 + g_1} \kappa C(x) \, dx, & \text{if } f > 1/2.
\end{cases}
\]

This equation holds for \( n > 0 \), but we must now address what happens when \( n = 0 \). As long as \( n = 0 \), we assume that the force is the isometric force (since the final steady-state velocity distribution must propagate out from \( x = h \) to \( x = L \)). This assumption is somewhat arbitrary, but we expect transient effects to be important in the \( n = 0 \) region, so for this short time period, the quasi-steady-state approximation will not be very good.

Then, using the following equation for the isometric steady-state force after stretch:

\[
F_{\infty} = \frac{1}{2\ell} \int_{0}^{h} \frac{\kappa N_{N_{1}h} \left( x, f_{1} \right) f_{1}^{2}}{f_{1}^{2} + g_{1}^{2}} \, dx,
\]

where \( n \) is the integer part of \( (h + \ell + L)/2 \) and \( f \) the fractional part. This definition is the same as the previous one, since \( \ell = \ell \). We may write an expression for force enhancement:

\[
F_{E} \approx \begin{cases} 
0, & n = 0, \\
\Delta F_{\text{iso}} N_{0} \left( 1 - (1 - a)^{-1} \right), & f < 2, \\
\Delta F_{\text{iso}} N_{0} \left( 1 - (1 - a)^{-1} \right) \left( 1 - a \left( \frac{a^{1/2} - 1}{a^{1/2} - 1} \right) \right), & f \approx 2, \\
\Delta F_{\text{iso}} N_{0} \left( 1 - (1 - a)^{-1} \right), & f > 2.
\end{cases}
\]

References