

# Microscale velocity-dependent unbinding generates a macroscale performance-efficiency tradeoff in actomyosin systems

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I can be brief in this review: I think this paper is terrific, well-written, interesting and compelling results, carefully constructed. My major issue is that I don't see how the Hillbot robophysical model contributes to the main biological point (about the potential optimality of  $\alpha$ ). I don't mean to say that it is not important or cool. I just think that "selling" the robot as a model of the system isn't quite right: the same results could have been obtained (I think) by integrating the motor equations of motion. I think the real value of the robophysical model is showing how the results of biology can influence new robot actuator design (and principles). Thus if I might be so bold I would reframe the robot aspect of the story as an instantiation of the principle to show it can work in the "real world". Otherwise, the robophysical model isn't really acting as a model (or the test of a hypothesis). If the authors can do this (and it shouldn't be too hard), I think the paper should be accepted.

Reviewer #2

(Remarks to the Author)

The paper shows the existence of a tradeoff between performance and efficiency in actomyosin dynamics, largely influenced by the myosin velocity-dependent unbinding from the thin filament. The authors investigate the properties of the energetic tradeoff by combining an analytical microscale 2-state actomyosin model with a macroscale robot-physical muscle model, HillBot.

The 2-state model's simplicity is justified. The geometry, kinetics, and physics underpinning both models are explained in detail. The experimental design is robust. This study represents an important contribution not necessarily restricted to the muscle physiology field as highlighted in the discussion.

However, some aspects require further investigation.

The authors declare that the results from the 2-state model elucidate the molecular basis for the efficiency-performance trade quantitatively. They also declare that the same results are in strong agreement with in vivo muscle data taken from the literature. However, in Figure 2c, the deviation of the 2-state model from the in vivo data is consistent. A statement that clarifies the origin of this deviation or a statistical test that shows that is not significant (if it is the case) would better justify the conclusions that are made.

The same approach would be beneficial for the deviation between the electromechanical model and HillBot data in Figure 3h.

The detailed mathematical approach and the extensive bibliography cited made the study strong, but the novelty of the work is easily lost in the huge amount of information. Therefore, the paper would be more impactful if the authors better emphasized what is the novelty.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I thank the authors for the careful responses and recommend publication. Very nice work!

Reviewer #2

(Remarks to the Author)

In this new version of the manuscript, the authors successfully addressed all the points made in the first review. I don't have additional comments or concerns.

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# Referee Comments for Nature Communications Biology

Jake McGrath and José Alvarado

February 25, 2025

We sincerely appreciate the time and effort that the reviewers and editor have dedicated to evaluating our manuscript. In the following, we provide a point-by-point response to each comment addressing the concerns of the referees. To facilitate review, we have highlighted and labeled the corresponding changes in the revised manuscript using different colors based on the comment we are addressing. Below, we specify which color highlight pertains to each comment. Our labeling convention follows the format [Ref. X.Y] where we respond to referee X about their comment Y. Once again, we thank the reviewers for their valuable insights that have significantly strengthened our manuscript.

The document is structured as follows: Each numbered bullet point represents a specific comment or concern from a referee (organized by referee 1 and referee 2). Our corresponding response follows immediately as an indented bullet point where the corresponding changes to the manuscript are highlighted in the specified color and labeled as [Ref. X.Y].

## 1 Referee Comments

### Referee 1 – Robotics Expert

1. I can be brief in this review: I think this paper is terrific, well-written, interesting and compelling results, carefully constructed.

- We sincerely thank the reviewer for their positive evaluation of our manuscript, and we are excited to share our findings with the community.

2. My major issue is that I don't see how the HillBot robophysical model contributes to the main biological point (about the potential optimality of  $\alpha$ ). I don't mean to say that it is not important or cool. I just think that "selling" the robot as a model of the system isn't quite right: the same results could have been obtained (I think) by integrating the motor equations of motion.

- We thank the reviewer for their comment. While we agree that the electromechanical model (motor equations) is valuable in its own right, we believe HillBot plays a crucial role in validating our approach. HillBot serves as the physical instantiation of the physical mechanisms represented in our model, confirming that our model captures the correct underlying dynamics (as evidenced by the strong agreement between HillBot and the model in Fig. 3). Without this experimental counterpart, we could not confidently assert the relationship between nonlinearity, energy consumption, and power generation in a real physical system. Moreover, HillBot reveals emergent dynamics that are not immediately apparent from the equations alone, particularly the presence of two distinct timescales. This difference manifests

in the slight disagreement between HillBot and the electromechanical model in Fig. 3d and 3h. HillBot applies a voltage that drives motion on a fast timescale, while the controller measures and corrects this motion on a slower  $\sim 10$  ms timescale. These corrections cause a characteristic voltage drop and recovery (Fig. 3g), a "letting-go" mechanism reminiscent of how myosin motors disengage in our two-state model. The electromechanical model, by contrast, lacks an explicit separation of these timescales, leading to a more abrupt voltage drop and recovery (Fig. 3d). Notably, the controller's correction timescale in HillBot is of the same order as the period of myosin's power stroke ( $\sim 10$  ms). This raises a few unanswered questions for future research: is there a separation between action and feedback in biology? How does a delay in measurement affect feedback in biological systems? In summary, HillBot serves as a crucial bridge between theory and experiment, both validating our model and uncovering a key timescale separation that might otherwise be overlooked in theoretical analysis alone. To clarify the importance of HillBot, we have added text to the Results and Discussion sections of the manuscript, highlighted in yellow. We appreciate the referee for bringing up this concern, as these additions further strengthen our argument and reinforce the justification for incorporating experiments.

3. I think the real value of the robophyscial model is showing how the results of biology can influence new robot actuator design (and principles). Thus if I might be so bold I would reframe the robot aspect of the story as an instantiation of the principle to show it can work in the "real world". Otherwise, the robophysical model isn't really acting as a model (or the test of a hypothesis). If the authors can do this (and it shouldn't be too hard), I think the paper should be accepted.

- We appreciate the reviewer's comments and agree that HillBot cannot be used to test the validity of a purely biological hypothesis of muscle. We also agree with the reviewer that HillBot acts as an instantiation of a biological principle inspired by muscle dynamics and demonstrates that this instantiation can work in the real world. However, we do believe that experiments with HillBot do provide scientific value in validating a more general, physical hypothesis: specifically, that a nonlinear coupling between actuation and the resulting dynamics controls an energy-efficiency tradeoff.

Although our electromechanical model validates this physical hypothesis theoretically, HillBot experimentally confirms that the performance-economy tradeoff is an inherent consequence of Hill's nonlinear force-velocity curve. This is a striking result: despite the fundamental differences in how muscles and robots consume energy and produce work, when actuated under Hill's force-velocity curve, governed by  $\alpha$ , we recover the same tradeoff between performance and economy. The fact that we observe this tradeoff (and the resulting optimum of  $\alpha \sim 4$ ) in both muscle and HillBot suggests that similar nonlinear couplings in other actuators could yield similar energetic tradeoffs and optima. Thus, HillBot serves to demonstrate the generality of the physics uncovered in our model.

In particular, Fig. 4e emphasizes this generality: decreasing power with linear actuation (blue circles) does not result in efficiency gains; only when we decrease power via the introduction of a nonlinear coupling through  $\alpha$  (orange circles) do we observe gains in efficiency. This experiment is impossible to perform with intact muscle and demonstrates the importance of performing experiments with HillBot. We have updated the caption of Fig. 4 to

further highlight this result. We sincerely appreciate the reviewer’s perspective. To address their comment, we have added text to the manuscript, **highlighted in green**, emphasizing HillBot’s role in demonstrating that nonlinear coupling between actuation and the resulting dynamics governs an energy-efficiency tradeoff. And, importantly, the nonlinear coupling alone generates this tradeoff, as illustrated in Fig. 4. We are grateful to the referee for raising this concern as these additions further strengthen our argument for integrating HillBot.

## Referee 2 – Actomyosin Expert

1. The paper shows the existence of a tradeoff between performance and efficiency in actomyosin dynamics, largely influenced by the myosin velocity-dependent unbinding from the thin filament. The authors investigate the properties of the energetic tradeoff by combining an analytical microscale 2-state actomyosin model with a macroscale robot-physical muscle model, HillBot. The 2-state model’s simplicity is justified. The geometry, kinetics, and physics underpinning both models are explained in detail. The experimental design is robust. This study represents an important contribution not necessarily restricted to the muscle physiology field as highlighted in the discussion.

- We thank the referee for their concise and accurate summary of our results, and we appreciate their recognition of our work’s broader relevance beyond muscle physiology.

2. However, some aspects require further investigation. The authors declare that the results from the 2-state model elucidate the molecular basis for the efficiency-performance trade quantitatively. They also declare that the same results are in strong agreement with in vivo muscle data taken from the literature. However, in Figure 2c, the deviation of the 2-state model from the in vivo data is consistent. A statement that clarifies the origin of this deviation or a statistical test that shows that is not significant (if it is the case) would better justify the conclusions that are made.

- We thank the reviewer for their comment; the reviewer rightly points out the deviation of our model from the in-vivo data in Fig. 2c (particularly in the high- $\alpha$  regime). This deviation is the same deviation shown in Fig. 1b and 1c. To explain the deviation, we first explain how we calculate Fig. 2c. To calculate  $PC$  in Fig. 2c for a given  $\alpha$ , we take the actuator’s FV curve (Fig. 1b) and integrate it by Eq. 9 which gives a scalar between 0 (weak actuator) and 1 (strong actuator). The model is initiated with the myosin fully attached  $A_0 = 1$ , therefore, when the model begins to contract for small contraction rates, the myosin are still mostly attached and producing large forces. Therefore, as shown in Fig. 1b and 1c, the model consistently slightly over predicts forces at low contraction rates (where this effect is amplified for larger  $\alpha$  because these curves drop off in force production quicker). When integrated, this consistent slight over prediction in force at small contraction rates explains why the model predicts larger  $PC$  than the in-vivo data.

Our goal with the simple two-state model is to construct a minimalist model free of fit parameters that accurately captures the essence of Hill’s nonlinear form and the in-vivo data. In our first approach, through an order of magnitude approximation, we estimated the parameters  $\beta = \gamma = \zeta = 1$  where  $\beta$  relates muscle actuation properties to the inertia of the

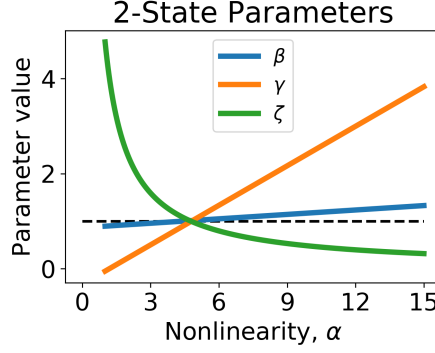


Figure 1: Model parameters  $\beta(\alpha)$ ,  $\gamma(\alpha)$ , and  $\zeta(\alpha)$  vary as a function of  $\alpha$ . Black-dashed line indicates 1.

attached mass,  $\gamma$  is a steady-state detachment rate, and  $\zeta$  relates the rate of Gibbs free-energy release due to myosin ATPase activity to the rate of mechanical work output.

However, these parameters can be written in terms of the nonlinearity  $\alpha = K_D/k_a$  such that  $\beta = \frac{f_0}{mv_0k_a} = \frac{f_0}{mv_0K_D} \cdot \alpha$ ,  $\gamma = \frac{k_0}{k_a} = \frac{k_0}{K_D} \cdot \alpha$ , and  $\zeta = \frac{k_ag_0}{f_0v_0} = \frac{K_Dg_0}{f_0v_0} \cdot \frac{1}{\alpha}$ . In this respect, we can write  $\beta(\alpha) = b \cdot \alpha$ ,  $\gamma(\alpha) = g \cdot \alpha$  and  $\zeta(\alpha) = z/\alpha$  where  $b, g, z$  become fit parameters. Each parameter,  $\beta(\alpha)$ ,  $\gamma(\alpha)$ , and  $\zeta(\alpha)$ , are still of order 1, but now can take on a value of up to 5 for the range of  $\alpha$  tested. Through this approach, we can construct a model with much better fit to Hill's equation.

Below in Table 1, we quantify the model's agreement with Hill's equation for different  $\alpha$  values by two different fit metrics through our first approach of setting  $\beta = \gamma = \zeta = 1$ . Then, in Table 2, we show an improved fit when allowing  $\beta(\alpha)$ ,  $\gamma(\alpha)$ ,  $\zeta(\alpha)$  to vary with  $\alpha$ . Figure 1 shows how  $\beta(\alpha)$ ,  $\gamma(\alpha)$ , and  $\zeta(\alpha)$  vary as a function of  $\alpha$ .

| $\alpha$ | 1    | 2    | 5    | 10   |
|----------|------|------|------|------|
| $R^2$    | 0.93 | 0.97 | 0.91 | 0.68 |
| $RMSE$   | 0.04 | 0.03 | 0.03 | 0.05 |

Table 1: Agreement of fit ( $R^2$  and  $RMSE$ ) between the model's FV curve and the predictions of Hill's equation when  $\beta = \gamma = \zeta = 1$ . For low  $\alpha$ , the model fits well. As  $\alpha$  becomes highly nonlinear, the fit weakens. Small overpredictions from the model compound in the integral in Eq. 9, leading to larger  $PC$  values.

| $\alpha$ | 1    | 2    | 5    | 10   |
|----------|------|------|------|------|
| $R^2$    | 0.99 | 0.98 | 0.94 | 0.83 |
| $RMSE$   | 0.02 | 0.02 | 0.03 | 0.04 |

Table 2: Agreement of fit ( $R^2$  and  $RMSE$ ) between the model's FV curve and the predictions of Hill's equation when  $\beta(\alpha)$ ,  $\gamma(\alpha)$ ,  $\zeta(\alpha)$ . Model accuracy is improved when parameters are allowed to vary with  $\alpha$ ; each parameter is still of order 1.

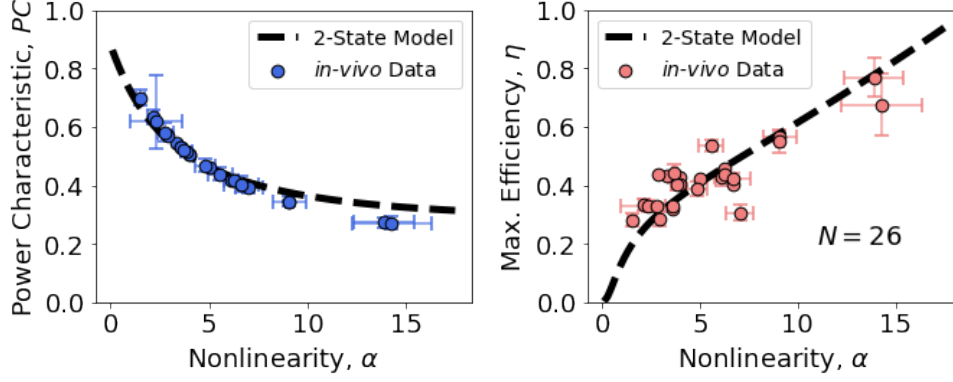


Figure 2: (a)  $PC$  predictions from the model with in-vivo data, and (b)  $\eta$  predictions from the model with in-vivo data. Allowing  $\beta, \gamma, \zeta$  to be function of  $\alpha$  leads to a much stronger fit to the in-vivo data.

By allowing  $\beta(\alpha), \gamma(\alpha), \zeta(\alpha)$  to be functions of the nonlinearity  $\alpha$ , we find much stronger agreement between our model and the in-vivo data as shown in the update panels of Fig. 2c and 2d below in Figure 2.

To address the reviewers concern, we updated our model in the manuscript to include the information in this response (namely, we changed  $\beta(\alpha), \gamma(\alpha), \zeta(\alpha)$  instead of limiting  $\beta = \gamma = \zeta = 1$  to find stronger agreement with the experimental in-vivo data). Moreover, we added the above Figure 1 to the SI with supporting text outlining how to write  $\beta(\alpha), \gamma(\alpha), \zeta(\alpha)$ . Changes addressing this comment are highlighted in light blue in the manuscript. We thank the reviewer for their comment as this change significantly strengthens the fit between our model and the in-vivo muscle data.

3. The same approach would be beneficial for the deviation between the electromechanical model and HillBot data in Figure 3h.

- We thank the reviewer for their comment; the reviewer points out the difference in efficiency predictions between HillBot and electromechanical model (Eq. 10-13). The deviation in Fig. 3h originates from Fig. 3d which is derived from a separation of timescales present in HillBot that is not present in the electromechanical model.

HillBot applies a voltage that drives motion on a fast timescale, while the controller measures and corrects this motion on a slower  $\sim 10$  ms timescale. These corrections cause a characteristic voltage drop and recovery (Fig. 3g), a "letting-go" mechanism reminiscent of how myosin motors disengage in our two-state model. The electromechanical model, by contrast, lacks an explicit separation of these timescales, leading to a more abrupt voltage drop and recovery (Fig. 3d). This quick, large drop in voltage with a slow recovery causes the model to over predict efficiency as it overall draws less electrical power while producing the same mechanical outputs of HillBot (Fig. 3e, 3f).

A longer, outer timescale is present in HillBot as measurements take  $\sim 10$  ms to record and process, whereas in the electromechanical model, the equations are coupled and thus integrated forward in time together. This leads to one timescale in the model; the integration time-step  $dt \sim 0.1$  ms.

If our goal were to construct the most precise and accurate model, it would require a more complex set of differential equations that accounts for this timescale separation. However, our aim is to construct the simplest possible model that faithfully captures the essential experimental behavior with minimal assumptions.

Because this separation of timescale is an important feature that requires attention, we added a few lines of text in the Results and Discussion section, highlighted in yellow, to address this separation of timescales in HillBot that is not present in the electromechanical model.

We would like to point out that the overestimation in efficiency from the electromechanical model is still small enough to allow us to predict the mechanics of HillBot accurately in Fig. 3e, 3f. Importantly, the data and model shown in Fig. 3h still support our claim: as the nonlinearity  $\alpha$  increases, the actuator's  $PC$  decreases and its efficiency  $\eta$  increases. We thank the reviewer for bringing the deviation in Fig. 3h to our attention; the added text highlighted in yellow discussing this separation of timescales in HillBot provides an explanation to the reader on the origin of the deviation between model and experiment. We believe that this added discussion on a separation of timescales between action and control could initiate potential future research directions.

4. The detailed mathematical approach and the extensive bibliography cited made the study strong, but the novelty of the work is easily lost in the huge amount of information. Therefore, the paper would be more impactful if the authors better emphasized what is the novelty.

- We appreciate the reviewer's perspective and recognize that the depth of information in our submission may obscure the novelty of our work. To better emphasize our key contributions, we made several targeted revisions highlighted in orange.

First, we added a concluding section to the Results (2.4) that concisely summarizes the novelty of our findings. In this section, we introduce Table 1, which clearly presents our main result: the most commonly observed  $\alpha^*$  optimizes a tradeoff between efficiency and power in both the two-state model and HillBot.

Secondly, we streamlined our abstract to remove unnecessary details and sharpen the focus on our novel contributions. Specifically, we now emphasize our key findings highlighted in Results 2.4: the most frequently observed value of  $\alpha^*$  across a wide range of muscles and species aligns with a nonlinearity that minimizes a power-efficiency tradeoff in both our model and experimental system.

Lastly, we expanded the discussion to further highlight how HillBot can be extended to future studies, reinforcing its role as a novel experimental platform for investigating biological principles in a controlled, mechanistic framework. We appreciate the reviewer's feedback, as these changes, highlighted in orange, emphasize the novelty of our work.