Metabolic Buffer Analysis Reveals the Simultaneous, Independent Control of ATP and Adenylate Energy Ratios

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Abstract

Determining the underlying principles behind biological regulation is important for understanding the principles of life, treating complex diseases, and creating *de novo* synthetic biology. Buffering - the use of reservoirs of molecules to maintain molecular concentrations - is a widespread and important mechanism for biological regulation. However, a lack of theory has limited our understanding of its roles and quantified effects. Here, we study buffering in energy metabolism using control theory and novel buffer analysis. We find that buffering can enable the simultaneous, independent control of multiple coupled outputs. In metabolism, adenylate kinase and AMP deaminase enable simultaneous control of ATP and adenylate energy ratios, while feedback on metabolic pathways is fundamentally limited to controlling one of these outputs. We also quantify the regulatory effects of the phosphagen system - the above buffers and creatine kinase - revealing which mechanisms regulate which outputs. The results are supported by human muscle and mouse adipocyte data. Together, these results illustrate the synergy of feedback and buffering in molecular biology to simultaneously control multiple outputs.

Introduction

Determining the design principles behind biological regulation is important for understanding the principles of life, treating complex diseases, and creating *de novo* synthetic biology [20]. A significant amount of research into this topic has focussed on feedback regulation [2, 8, 9, 18, 20, 32, 33, 34, 40], a ubiquitous mechanism in biology that acts via biological actuators, such as regulated enzymes [17, 35]. But there is a crucial open challenge to determine the underlying principles for biological regulation beyond feedback [20]. Buffering—the use of reservoirs of molecules to maintain molecular concentrations—is another widespread mechanism for biological regulation [17, 35]. Despite its importance, buffering has received considerably less attention than feedback. In recent work, we studied the interaction of feedback and buffering, where we found that synergies between them are often critical for biochemical regulation [17, 16]: Feedback regulates 'slow' disturbances while buffering regulates 'fast' disturbances and stabilises feedback.

However, we do not know all of the underlying principles of buffers and have a limited methodology for quantifying their effects. Our recent findings describe the principles where there is buffering of a single regulated output. While important, they do not fully describe cases where buffers affect multiple outputs, such as in energy metabolism [5]. Also, the familiar treatment for quantifying the effects of buffering is limited to closed systems (with mass and energy isolated from surroundings), whereas *in-vivo* biological systems are fundamentally open systems [20]. Further, the methodology is heavily focussed on pH buffering, while biological systems are predominantly non-pH buffers. Thus, there is a requirement for novel theory relating buffering to

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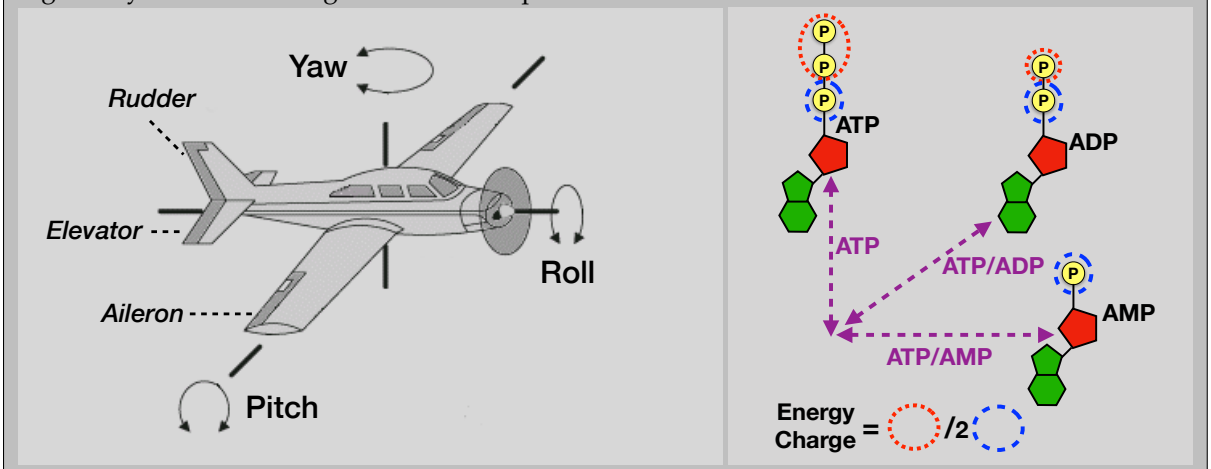
Box 1: Multivariable Control in an Energy Metabolism Context

In control theory, the simultaneous, independent control of multiple coupled outputs is referred to as 'multivariable control'. It is an important topic that has been extensively studied in technology and other non-biological contexts [36], but not in biological ones. However, the control of multiple coupled outputs is also important in biology, such as in energy metabolism. In this paper, we provide a foundational case study for multivariable control in cellular biology.

To help describe multivariable control in an energy metabolism context, we can compare the control of an aircraft's (rotational) direction with that of the adenine nucleotide energy environment of a cell. We need to use three variables to describe the direction of an aircraft; roll, pitch and yaw (see below figure [38]). Without all three, we don't have a complete picture of the aircraft's direction. To describe the adenylate energy environment of a cell, we similarly need to use three variable as there are three molecules involved (ATP, ADP and AMP). The important outputs are ATP, the ATP/ADP ratio, the ATP/AMP ratio and the energy charge (see introduction for descriptions), noting that one is redundant when all are used in combination.

To control the direction of an aircraft or energy environment of a cell, the three outputs all need to be simultaneously controlled. For an aircraft, the rudder (yaw), ailerons (roll), and elevators (pitch) are the regulatory mechanism that carry out the control of each of these outputs. For energy metabolism, the energy environment is controlled by the reaction rates of the metabolic pathways as well as creatine kinase, adenylate kinase and AMP deaminase.

In the below analysis, we first show that these buffers are required to simultaneously and independently control the three outputs. We then quantitatively characterise the buffers to show which regulatory mechanisms regulate which outputs.



multiple outputs, biochemical kinetics, and the quantification of regulation in open systems.

To understand regulatory roles in the case of energy metabolism, we need to know what outputs are regulated by which mechanisms from what types of disturbances [4, 17]. Disturbances, such as changing energy demand, cause deviations in the outputs due to a mismatch of ATP demand and supply, while the regulatory mechanisms reduce these deviations. Studies focus on the following key outputs: ATP concentration, which is the cellular energy currency; and the ATP/ADP and ATP/AMP concentration ratios, which are related to Gibbs free energy, i.e., the ability of a cell to do work (also see Box 1). These quantities are analogous to charge (ATP) and voltage (free energy) on batteries. Studies also focus on the adenylate energy charge $(ATP + 0.5ADP)/(ATP + ADP + AMP)$, an index used to measure the overall energy status of biological cells [5] (see figure in Box 1). A widespread view is that cells primarily regulate for the energy charge or related AMP-based outputs, which is based on the energy charge's steady dynamics and the observation that signalling and allosteric feedback primarily regulates for these outputs [5].

Despite extensive studies on metabolic buffers, a lack of theory has limited our understanding of

their roles and quantified effects. This limitation is particularly important for understanding the phosphagen system [6], a critical buffering system in energy metabolism that is composed of adenylate kinase (AK), AMP deaminase (AMPD), and creatine kinase (CK). In the phosphagen system, creatine kinase regulates for fast-changing disturbances and stabilises glycolytic feedback [17, 39], while AK and AMPD are buffering enzymes that have a number of regulatory roles relating to different energy outputs [5, 25, 29]. AK catalyzes the regeneration of ATP from ADP and enables AMP-based feedback by also producing AMP [5, 25]. AMPD removes AMP and limits its accumulation [5]. It has been proposed that AMPD's role is to regulate adenylate ratios [5]. Other studies propose that AK and AMPD buffering is intended to limit the accumulation of ADP in muscles [15, 14]. However, it is difficult to collectively interpret the phosphagen system's many roles without understanding the underlying principles, or to gauge their relative importance without quantification.

Here, we study buffering in energy metabolism using control theory and novel buffer analysis for open systems. We find that buffering can enable the simultaneous, independent control of multiple coupled outputs, which is in addition to its known role of regulating 'fast' disturbances. In energy metabolism, we find that adenylate kinase and AMP deaminase enable the simultaneous control of ATP and the key adenylate energy ratios, whereas feedback on metabolic pathways is fundamentally limited to controlling one of these outputs. This result significantly differs from, but can also explain, the widespread view that cells primarily regulate for a single adenylate energy output. We also quantify the regulatory effects of the phosphagen buffering system to reveal which mechanisms regulate which outputs. We find that AK regulates the ATP/ADP ratio while AMPD regulates the ATP/AMP ratio and the energy charge. We find that creatine kinase is significantly more effective when the ATP/ADP ratio is highly sensitive to changes. The phosphagen system quantification is shown to match with human muscle and mouse adipocyte data, including with AK knockout data. Together, these results illustrate the synergy of feedback and buffering in cellular biology to simultaneously control multiple outputs.

We use two separate but complementary methods in the paper: In section 1, we qualitatively study the independent control of multiple energy outputs using control theory, and in section 2-5, we quantify the regulatory effect of the phosphagen system using novel buffer analysis.

1 Controllability: The Simultaneous Control of ATP and Adenylate Energy Ratios

In this paper, we are interested in studying buffering and its interaction with feedback. In particular, we focus on the phosphagen system [6] and its interaction with feedback on metabolic pathways. The phosphagen system can be represented by the buffering reactions

$$ATP + Cr \leftrightarrow pCr + ADP \quad \text{(creatine kinase)} \quad (1)$$
$$ATP + AMP \leftrightarrow 2ADP \quad \text{(adenylate kinase)} \quad (2)$$
$$AMP \rightarrow IMP + NH_4 \quad \text{(AMP deaminase)} \quad (3)$$

where ATP, ADP and AMP are the adenine nucleotides used for energy, pCr is creatine phosphate, Cr is creatine, and IMP is inosine monophosphate. IMP can be regenerated to form AMP in separate reactions. The right hand side of (1) and the left hand side of (3) can also include H^+, but we do not include them here both for simplicity and so that we can later use standard definitions of equilibrium constant K.

Here, we ask what concentrations and outputs can feedback on metabolic pathways and the phosphagen system control? This question is well suited to controllability analysis from control theory [4]. Controllability determines the ability for regulatory mechanism inputs (e.g. enzyme activity) to change the system states (concentrations). Determining the actions of regulatory mechanisms contrasts to determining the outputs the mechanism responds to or senses. For example, a pathway enzyme may respond to a change in AMP by acting on ATP production, where controllability analyses the action on ATP and not what is sensed.

To model regulatory mechanisms, we assume that the reaction AMP→ADP occurs via adenylate kinase and that regeneration of ATP, ADP and AMP from other sources occurs via the regeneration of AMP from IMP (see SI1 for models). Other regeneration reactions also occur in cells, such as the production of AMP from the degradation of RNA, where these reactions are incorporated in

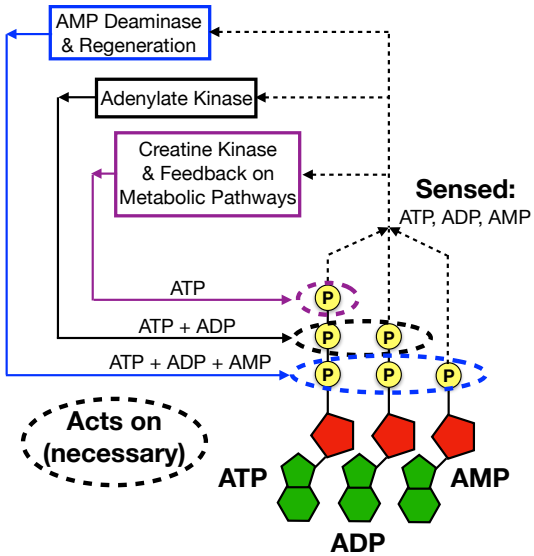


Figure 1: Representation of the control actions of the phosphagen system and feedback on metabolic pathways.

the models as disturbances rather than energy regulation mechanisms. We do not make assumptions regarding ATP consumption and the models incorporate all forms of these processes. Below, we discuss the case incorporating energy buffers that are closely related to the phosphagen system, showing equivalent results.

For clarity of results, we also assume that the reactions all occur in a single compartment. Without this assumption, we would need to model the phosphagen system and corresponding adenine nucleotide concentrations in multiple compartments, which would unnecessarily complicate analysis without providing greater insight.

To simplify analysis, we describe the system in terms of the total adenine nucleotide phosphates in the third (ATP), second (ATP+ADP) and first (ATP+ADP+AMP) phosphate groups (see Figure 1 and SI1), also referred to as the γ, β and α phosphates respectively.

We first consider the case without AK (see SI1 for controllability analysis). We need to ensure a steady state at non-zero concentrations and so for this case we also have to ignore any reactions that cannot be regenerated without AK, such as ATP$\rightarrow$AMP and ADP$\rightarrow$AMP. Without AK, feedback on pathways (controlling ADP$\rightarrow$ATP and ATP$\rightarrow$ADP) cannot independently control both ATP and the ATP/ADP ratio as ATP+ADP is constant i.e. unable to be controlled. For a given value of ATP, then ADP is immediately determined, which holds no matter what the feedback network senses or how complex the network is. To simultaneously and independently control both, or in fact any two independent combinations of ATP and ADP, the total ATP+ADP also needs to be controlled. Adenylate kinase is able to ensure that the system is controllable as it varies the total ATP+ADP, and thus can enable the control of ATP and the ATP/ADP ratio (See Figure 1). Similar to feedback on metabolic pathways, creatine kinase also has no ability to control the total ATP+ADP.

To determine the effect of AMP deaminase, we study the system with adenylate kinase but without AMP deaminase or its corresponding AMP regeneration from IMP. We need to ensure a steady state at non-zero concentrations and so for this case we also have to ignore any processes that cannot be regenerated via resynthesis of IMP. Similar to above, ATP, ATP/ADP and the ATP/AMP ratio, or any three independent combinations of ATP, ADP and AMP, cannot be simultaneously controlled without AMP deaminase (see SI1 for analysis and Figure 1 for a graphical representation). This lack of controllability results from a constant ATP+ADP+AMP i.e. it is unable to be controlled. AMP deaminase is able to enable simultaneous control of independent combinations of ATP, ADP and AMP as it varies the total ATP+ADP+AMP.

Thus we can observe that adenylate kinase and AMP deaminase enable the simultaneous, independent control of ATP, ADP and AMP (and thus all of the energy ratios). In contrast, the system with feedback on metabolic pathways but without buffering is not controllable and is fundamentally limited to only controlling one of these outputs.

Above, we assumed that ADP$\rightarrow$ AMP occurs via adenylate kinase, which is the dominant path [7]. However, the reaction can also occur through buffering reactions that are closely related to the phosphagen system, such as specific forms of polyphosphate kinase [1], where polyphosphate carries out an analogous role to creatine phosphate in microorganisms. For this case, the results still hold if we group these enzymes with adenylate kinase (see SI1 for analysis), just as we grouped creatine kinase with feedback on metabolic pathways (See Figure 1). It is also possible for buffering reactions closely related to the phosphagen system to regenerate ATP, ADP, or AMP from other sources. Once again, the results still hold if we group these reactions with the reactions regener-

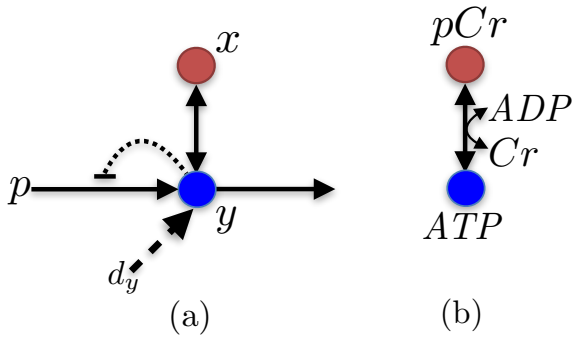


Figure 2: (a) Buffering and feedback schematic. x and y are the concentrations of the buffer and regulated species, respectively. p and d_y are the the production and disturbance rates of the regulated species. (b) An example with pCr buffering

ating AMP from IMP.

While the controllability analysis shows what the regulatory mechanisms can and cannot act on, it does not show which combination of ATP, ADP and AMP the particular mechanisms are regulating, or by how much, as it is a qualitative method that does not take into account what the mechanisms respond to (as discussed above). For this question, we need to quantify the regulatory effects of energy buffers.

2 Quantifying Buffering: Regulation Metrics in Open Systems

To quantify the regulatory effects of systems with feedback and buffering, we use two key measures of regulation: the sensitivity function and the buffer equilibrium ratio [17]. The sensitivity function quantifies the overall regulatory effectiveness produced by all regulatory mechanisms present (e.g., both feedback and buffering), while the buffer equilibrium ratio is a metric specific to buffering. The latter is independent from other forms of regulation and from the disturbance itself (see SI2.4 for analysis of a simple example).

The buffer equilibrium ratio compares the change in an output concentration, y, to the change in the concentration of a buffering species, x, when the buffering reactions are at equilibrium [17] (see Figure 2). This is written as

$$B = \frac{\Delta x}{\Delta y} \tag{4}$$

where Δy and Δx are the deviations of y and x from their undisturbed steady states ("set points") $\bar{y}$ and $\bar{x}$ (see SI2.1). Thus, B represents the degree to which the effects of the disturbance have been absorbed by the buffer vs permeated through to the system output. One use of B is to measure a buffers ability to regulate 'fast' disturbances [16, 17], where large B implies improved regulation (B also has an important control-theory interpretation in terms of derivative feedback [16, 17]; also see SI2.3). For small disturbances, B can be locally approximated about the system set point (see SI2.2). An equivalent approximation is used to define the well-known "buffer capacity" metric for pH regulation in closed systems (see SI3), and for steady-state sensitivity analysis as commonly applied in systems biology [32].

It is important to distinguish B from the familiar thermodynamic equilibrium constant, K: the latter relates (zero-disturbance) steady-state concentrations directly (e.g., $K = \bar{y}/\bar{x}$), while B relates their (non-zero disturbance) deviations from steady state.

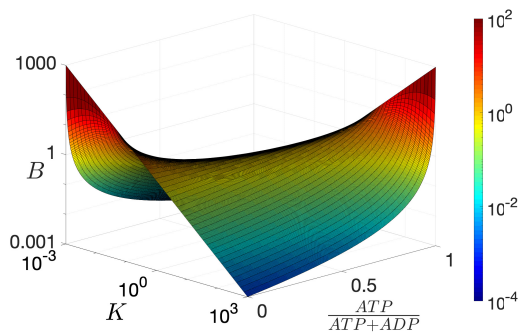
The sensitivity function is a normalised measure of the change in a system output, y, due to a disturbance, d_y. Again, it is affected by all internal regulatory mechanisms present. The sensitivity function is written as

$$\phi = \frac{\|\Delta y\|_t}{\bar{y}} \Big/ \frac{\|d_y\|_t}{\bar{p}} \tag{5}$$

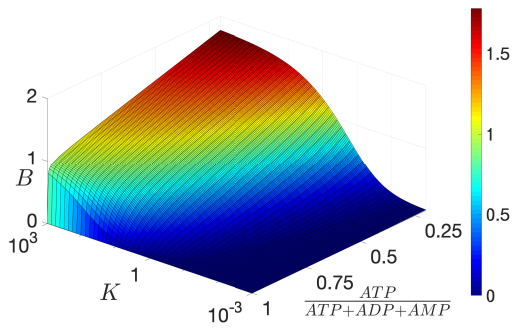
where $\|\Delta y\|_t$ represents the size of deviation Δy accumulated over time, $\|d_y\|_t$ represents the size of disturbance d_y accumulated over time, $\bar{y}$ is the set point, and $\bar{p}$ is the production rate of y at the set point. The exact form of $\|d_y\|_t$ and $\|\Delta y\|_t$ depends upon the type of disturbance. For example, for an oscillating disturbance both may reflect the amplitude of oscillations.

The sensitivity function (5) is closely related to commonly used sensitivity analysis in systems biology for studying steady states [32], noting that the function used here can incorporate temporal dynamics as well as steady-state analysis.

The reader may find it instructive to compare and contrast the analysis of pH buffering with open-system metrics introduced in Section 2 to familiar metrics commonly used to analyze closed-system, which can be found in SI3.



(a) Creatine Kinase



(b) Adenylate Kinase

Figure 3: Buffer Equilibrium Ratios for CK and AK, which quantify their buffering effects under different conditions. CK has normalisation $C/D = 1$ where $C = [pCr] + [Cr]$ and $D = [ATP] + [ADP]$.

3 Creatine Kinase

In this section, we quantify the specific effect of creatine kinase buffering under different conditions. To achieve this, we determine its buffer equilibrium ratio, which can measure its ability to regulate 'fast' disturbances. We find that creatine kinase is most effective at high K and high ATP/ADP ratios (or low K and low ATP/ADP ratios). We also show that the effectiveness of creatine kinase is reduced in human muscles by adenylate kinase. However, this is offset by the boost to the effectiveness of creatine kinase from varying equilibrium constant K (via changing pH). We show that the theory matches experimental data for human muscle during and after exercise.

The buffer equilibrium ratio for the creatine phosphate reaction described in equation (1) is

$$B_c = \frac{\Delta x_c}{\Delta y} = \frac{\Delta[pCr]}{\Delta[ATP]}.$$

If we initially assume that the equilibrium constant K_c and other parameters are fixed then (see SI4.2)

$$B_c^f = \frac{C}{D} \frac{K_c}{\left(K_c + (1 - K_c)\frac{ATP}{D}\right)^2} \tag{6}$$

where $x_c = [pCr]$, $y = [ATP]$, $C = [pCr] + [Cr]$, $D = [ATP] + [ADP]$, the equilibrium constant is $K_c = [ATP][Cr]/([pCr][ADP])$, and C, D and K_c are the fixed parameters. We can observe that the buffer equilibrium ratio B_c^f in (6) increases linearly with C/D, but the relationship between B_c^f, K_c and ATP/D is more complicated.

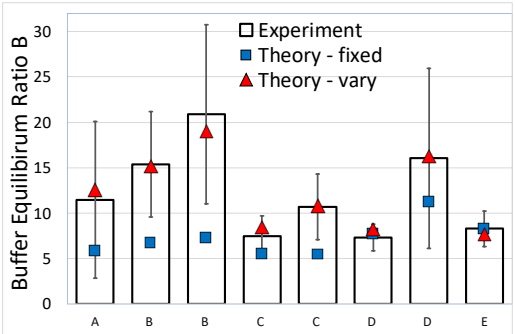
In Figure 3, we can see that there are two regions with large B_c^f, and consequently where pCr is most effective at regulating 'fast' disturbances. These regions occur where K_c is small together with small ATP/ADP, and where K_c is large together with large ATP/ADP, noting that ATP/ADP is large when ATP/D is close to 1. Outside of these regions B_c^f is significantly smaller and consequently the buffer is less effective.

In calculating B_c, we also need to take the varying equilibrium constant K_c and $D = ATP + ADP$ into account. For example, during exercise the use of ATP decreases intracellular pH, which increases the value of K_c. Similarly, parameter D decreases as ATP levels decrease due to adenylate kinase converting ADP to AMP. For varying K_c and D, the buffer equilibrium ratio of pCr is (see SI4.2)

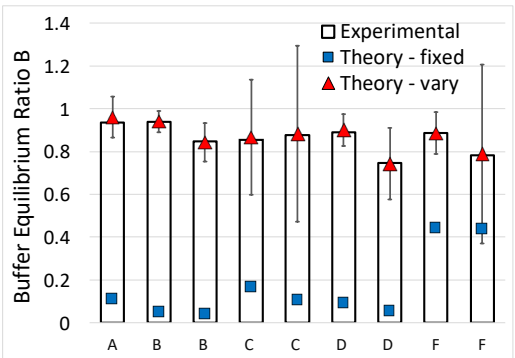
$$B_c = B_c^f \left(1 - \left[1 - \frac{ATP}{D}\right] \underbrace{\frac{ATP}{K_c}\frac{\Delta K_c}{\Delta ATP}}_{\text{K/pH}} - \underbrace{\frac{ATP}{D} B_a}_{\text{AK}}\right) \tag{7}$$

where B_c^f is given in (6), $B_a = \Delta D/\Delta ATP$ is the rate of change of $D = [ATP] + [ADP]$ with respect to ATP (also see section 4), $\Delta K_c/\Delta ATP$ is the rate of change of equilibrium constant K_c with respect to ATP, and total creatine $C = Cr + pCr$ in (6) is assumed constant. It can be observed that the second term is due to changing K_c (via changing pH) while the third term is due to adenylate kinase.

Under high energy demand situations like exercise, K_c typically increases when ATP drops (via decreasing pH), which shifts reaction (1) to the left back towards ATP and increases the counteracting effect of buffering ($\uparrow B_c$). In contrast, AK removes more ADP when ATP decreases, which shifts reaction (1) to the right away from ATP and reduces the counteracting effect of buffering ($\downarrow B_c$).



(a) Creatine Phosphate



(b) Adenylate Kinase

Figure 4: Comparison of Experimental and Theoretical Buffer Equilibrium Ratios with fixed and varying parameters. A-E show human muscle data and F shows mouse adipocyte data (See Appendix).

We can compare the above theory to experimental data from literature, where Figure 4 shows values of buffer equilibrium ratio B_c for human muscles during and after exercise (see SI4.2.3 for calculation methods). It can be seen that the theoretical values of B_c without AK or varying K_c are not consistent with experimental values, but match with experimental values when these are included (also see SI4.2).

We can observe that the experimental value of B_c for CK ranges from 7.3 to 21. The metric $1/(1+B_c)$ can act as a proxy for the attenuation of fast disturbances by CK (see SI2.4). Thus the results infer that CK reduces deviations from fast disturbances by an order of magnitude.

4 Adenylate Kinase

In this section, we quantify the specific effect of adenylate kinase buffering under different conditions. To achieve this, we determine its buffer equilibrium ratio, which can measure its ability to regulate 'fast' disturbances and which is also used in Section 5 to quantify its regulation of multiple energy outputs. We find that AK typically has a buffer equilibrium ratio that is slightly less than one, which implies that its importance for regulating for 'fast' disturbances is secondary and significantly smaller than creatine kinase. We also show that AMPD increases the effect of AK, although the increased ratio is still slightly less than one in the experimental data. We also show that the theory matches experimental data for human muscle and mouse adipocytes.

To study the buffering properties of the adenylate kinase reaction described in Equation (2), we set the output as $y = [ATP]$ and the buffer or reservoir as $x_a = D = [ATP] + [ADP]$. If we had instead set ADP as the buffer then the ATP hydrolysis $ATP \rightarrow ADP$ would be a buffering reaction alongside reaction (2). By setting $x_a = D$, then ATP hydrolysis does not simultaneously remove y and produce x_a and so is independent of the buffering reactions.

The buffer equilibrium ratio for adenylate kinase is

$$B_a = \frac{\Delta x_a}{\Delta y} = \frac{\Delta(ATP + ADP)}{\Delta ATP}. \tag{8}$$

If we initially assume that the equilibrium constant K and other parameters are fixed then (see SI4.3)

$$B_a^f = \frac{[AMP] + 2K_a[ADP]}{[ATP] + 2K_a[ADP]} \tag{9}$$

where $K_a = \frac{[ATP][AMP]}{[ADP]^2}$ and the adenine nucleotide total $A = ATP + ADP + AMP$ is a fixed parameter. Equation (9) can also be written in a more complicated form as an explicit function of two independent inputs K_a and ATP/A (see SI4.3). A related result for the alternate purpose of determining changes in AMP to changes in ATP can be found in [32, 11] - changes in AMP correspond to changes in $ATP + ADP$ if A is fixed (see above) but not if A is varying (see below).

We can observe in Figure 3 that buffer equilibrium ratio B_a^f is higher for higher K_a values.

However, B_a^f is not large (compared to creatine kinase) at high ATP/A and K_a, with a maximum at or near one. Although not shown in the figure, B_a^f can become large at very low energy charges where $AMP > ATP$ (see SI4.3).

We also need to take AMPD and corresponding regeneration into account by incorporating varying A. For this parameter-varying case, the buffer equilibrium ratio of AK is (see SI4.3)

$$B_a = B_a^f \left(1 + \underbrace{\frac{[ATP]}{[AMP] + 2K_a[ADP]} R_m}_{\text{AMPD}}\right) \tag{10}$$

where $R_m = \Delta A / \Delta ATP$ is the rate of change of A with respect to ATP due the removal of AMP by AMP deaminase and the corresponding AMP regeneration (see SI4.3 for the effect of changing K_a).

AMPD removes AMP as ATP decreases, which shifts reaction (2) to the left back towards ATP and increases the counteracting effect of buffering ($\uparrow B_c$).

We can compare the theoretical buffer equilibrium ratios to human muscle and mouse adipocyte experimental data. Figure 4 shows values of theoretical and experimental B_a. It can be seen that the theoretical values of B_a from (2) are not consistent with experimental values when AMPD is not incorporated, but match with experimental values when it is (also see SI4.3). It can be noted that the adipocyte data also requires the assumption that K varies. The effect of AMPD on AK can be seen in Figure 4 to be much larger in muscle than in adipocytes, but that the buffer equilibrium ratio is just below one in both cases.

Figure 4 shows that the AK buffer equilibrium ratio B_a is slightly less than one in human muscle during/after exercise, while the pCr ratio is $B_c = 7.3 - 21$ during/after exercise. As the total buffer equilibrium ratio for ATP is $B_{tot} = B_c + B_a$ [17], we can observe that AK is much less important than pCr in regulating for 'fast' disturbances.

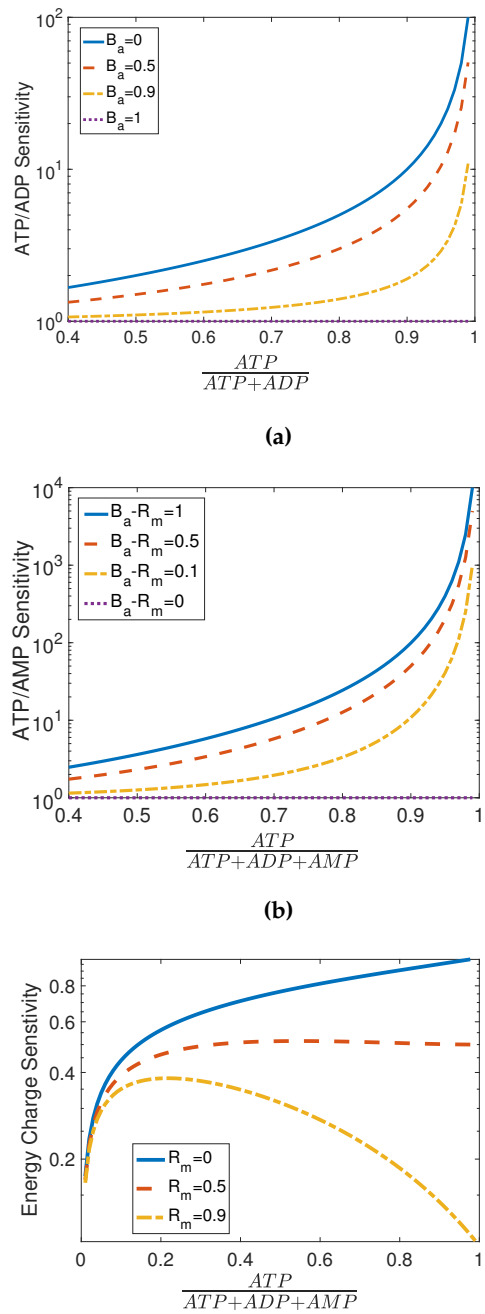


Figure 5: Sensitivity of Adenylate Energy Ratios to changes in ATP (as defined in (11)). These plots show the regulatory effect of AK (via B_a) and AMPD (via R_m) on different outputs.

5 Quantifying Ratio Regulation

In this section, we quantify the overall effect of the phosphagen system to regulate key energy outputs: the ATP/ADP ratio, the ATP/AMP ratio and the energy charge. We find that AK regulates the ATP/ADP ratio while AMPD regulates the ATP/AMP ratio and the energy charge. We also find that AK worsens regulation of the ATP/AMP ratio and the energy charge, but this tradeoff is compensated for by AMPD. The reader may recall that the ATP/ADP and ATP/AMP concentra-

tion ratios are related to Gibbs free energy, i.e. the ability of the cell to do work, while the adenylate energy charge $(ATP + 0.5ADP)/(ATP + ADP + AMP)$ is an index used to measure the overall energy status of biological cells [5]. We also show that the theory matches experimental data for human muscle and mouse adipocytes.

To quantify the regulation of the energy ratios, we determine the sensitivity of the energy ratios to changes in ATP. Determining these sensitivity functions enables a simple means of translating sensitivity function (5) for ATP into sensitivity functions for the energy ratios. We use ATP as a reference as the regulation metrics are most naturally written in terms of ATP concentrations e.g. disturbances are most naturally represented in terms of ATP demand or supply.

The sensitivity of the ATP/ADP ratio, ATP/AMP ratio and energy charge to changes in ATP are (see SI5.1)

$$\Delta_{\%}\left[\frac{ATP}{ADP}\right]\Big/\Delta_{\%}ATP = 1 + (1 - \underbrace{B_a}_{\text{AK}})\frac{ATP}{ADP}$$

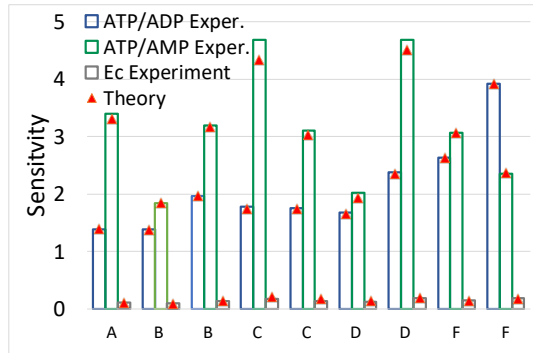
$$\Delta_{\%}\left[\frac{ATP}{AMP}\right]\Big/\Delta_{\%}ATP = 1 + (\underbrace{B_a}_{\text{AK}} - \underbrace{R_m}_{\text{AMPD}})\frac{ATP}{AMP}$$

$$\Delta_{\%}E_c\Big/\Delta_{\%}ATP = \left(1 + \underbrace{B_a}_{\text{AK}} - \underbrace{2E_C R_m}_{\text{AMPD}}\right)\frac{1}{2E_c}\frac{ATP}{A} \tag{11}$$

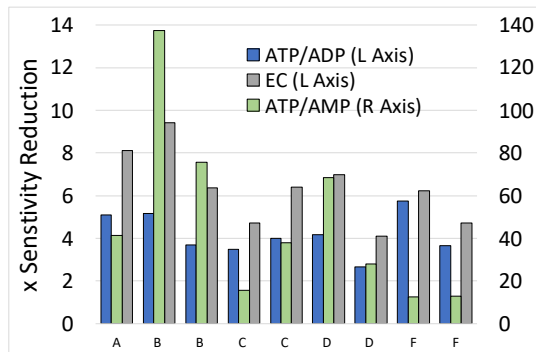
where $\Delta_{\%}\left[\frac{ATP}{ADP}\right]$, $\Delta_{\%}\left[\frac{ATP}{AMP}\right]$ and $\Delta_{\%}E_c$ are the percentage change in the respective ratios, $\Delta_{\%}ATP$ is the percentage change in $[ATP]$, B_a is defined in (8), R_m is defined below (10), A is defined below (9) and $E_c = (ATP + 0.5ADP)/(ATP + ADP + AMP)$.

Equation (11) is plotted in Figure 5 and shows that at high ATP/ADP or ATP/AMP ratio then small changes in ATP can result in large changes in both the ATP/ADP and ATP/AMP ratios repectively. This effect occurs as small relative changes in ATP cause large relative changes in ADP and AMP. The energy charge does not suffer from the same sensitivity effects as the other ratios (see Figure 5).

Equation (11) and Figure 5 show that AK reduces the sensitivity of the ATP/ADP ratio to changes in ATP, reaching a minimum when $B_a = 1$. AK also creates a tradeoff as increasing B_a worsens the regulation of the ATP/AMP ratio. However, AMPD regulates the ATP/AMP ratio by lessening this sensitivity effect, reaching a minimum when $R_m = B_a$. The regulation of the energy charge is also worsened by AK and improved by AMPD.



(a)



(b)

Figure 6: (a) Comparison of Experimental and Theoretical Sensitivity of ATP/ADP (left) , ATP/AMP (centre) and the Energy Charge (right) to changes in ATP. (b) Sensitivity Reduction of ATP/ADP by AK (left), ATP/AMP by AMP deam. (centre) and the Energy Charge by AMP deam. (right). A-E show human muscle data and F shows mouse adipocyte data (See Appendix).

This analysis effectively quantifies the regulation of the energy ratios into two forms. First, improving ATP regulation can be observed to improve regulation of the energy ratios, as reduced deviations in the former will reduce deviations in the latter (or vice versa). Through this general means, it is possible for improved regulation of ATP from feedback and pCr to also improve the regulation of the energy ratios (or vice versa). However, due to the sensitivity of the ratios un-

der some conditions, ATP may be well regulated while the energy ratios are poorly regulated. In the second form of regulation, AK and AMPD can reduce the sensitivity of the individual energy ratios to changes in ATP, enabling simultaneous effective regulation of ATP and the ratios. Interestingly, while pCr regulates 'fast' disturbances, AK and AMPD reduce the sensitivity of the energy ratios to any deviation in ATP. Thus AK and AMPD help regulate both 'fast' and 'slow' disturbances.

We can see in Figure 6 that the sensitivity functions match experimental data for human muscle and mouse adipocytes. The results in the figure show that on average AK reduces the ATP/ADP sensitivity by $\times 3.9/4.5$ (muscle/adipose), AMPD reduces the ATP/AMP sensitivity by $\times 37.8/\times 12.8$ (muscle/adipose) and AMPD reduces the energy charge sensitivity by $\times 6.1/\times 5.4$ (muscle/adipose).

The inferred regulation of the ATP/ADP ratio by AK is supported experimentally by AK knockout data, which resulted in a significant accumulation in ADP and worsening of the ATP/ADP ratio in skeletal muscle [14].

6 Discussion

The study of the regulatory roles of the phosphagen system illustrate an important general principle - buffering can enable the simultaneous, independent control of multiple coupled outputs. This role is in addition to its known role of regulating 'fast' disturbances and stabilising feedback. Different buffers can also carry out different roles. In the phosphagen system, AMPD and AK carry out the first role, while CK and AK (to a much lesser extent) carry out the latter roles. In AK's case, its ability to enable simultaneous control occurs due to its production and removal of AMP (and thus ATP+ADP), while its lesser second role occurs due to the regeneration of ATP. In an interesting difference between the roles, CK regulates 'fast' disturbances while AK and AMPD regulate both 'fast' and 'slow' disturbances.

In the buffer analysis presented, we have focussed on regulation (i.e. minimising deviations about a set point). However, the controllability analysis discussed in Section 1 is also applicable to control (i.e. changing the set point itself). The ability to simultaneously control different outputs can enable the energy environment to be optimised for different cells or compartments. AK controls the relationship between different ratios via $ATP/ADP = K_a ADP/AMP$, rather than explicitly controlling the ATP/ADP ratio. For this case, the set point of the ratios can be controlled by changing K_a e.g. by varying Mg^{2+}. This control is in conjunction with the other regulatory mechanisms to jointly control the ratios involving ATP, ADP and AMP.

Throughout this study, we have shown that multiple energy outputs are simultaneously regulated by different buffering and feedback mechanisms in a synergistic fashion. This finding differs from a general view that cells primarily regulate for one energy output, typically the energy charge or a related AMP-based output [5]. However, our findings are still consistent with this view if buffering is ignored as feedback on its own can only simultaneously control one adenylate energy output.

The quantification of metabolic buffers shows that the ATP/ADP and ATP/AMP ratios are highly sensitive to small changes in ATP when they are at high levels. It also shows how strongly the phosphagen system regulates these ratios when the ATP/ADP and ATP/AMP ratios are at high levels. Both creatine phosphate and adenylate kinase have large regulatory effects when the ATP/ADP ratio is highly sensitive to changes, while AMP deaminase has a large regulatory effect when the ATP/AMP ratio is highly sensitive to changes. Interestingly, this observation combined with our recent studies about the interaction of buffering and feedback [17, 16] would indicate pCr should also stabilise glycolytic feedback more strongly at high ATP/ADP and thus enable feedback to be more effective under these conditions. We can also observe in Figure 6 that while the energy charge (the standard energy output) has the 'steadiest' output, it is not necessarily the most strongly regulated. In human muscles, the ATP/AMP ratio is significantly more strongly regulated than the energy charge by AMPD, although they are more comparable in mouse adipocytes.

In control theory, the simultaneous, independent control of multiple coupled outputs is referred to as 'multivariable control' (see also Box 1), which is a topic that has been extensively studied in non-biological contexts [36] but not in biological ones. In this paper, we provide a foundational case study for multivariable control and cellular biology. As the methods are generic and both buffering and feedback are ubiquitous in biology, we believe that this topic will have significantly wider

applicability than the case study provided.

7 Conclusion

In this paper, we used control theory and novel buffer analysis for open systems to study buffering in energy metabolism. This enabled us to both uncover underlying principles in metabolic regulation and to quantify the effects of critical regulatory mechanisms. We showed the importance of adenylate kinase along with AMP deaminase and its regeneration for simultaneous, independent control of ATP and the adenylate energy ratios. We also quantified their effect and showed that AK regulates the ATP/ADP ratio while AMP deaminase regulates the ATP/AMP ratio and the energy charge. Similarly, we quantified the effect of creatine phosphate, and showed that it is significantly more effective when the ATP/ADP ratio is highly sensitive to changes. The results were shown to be consistent with experimental data for human muscle and mouse adipocytes.

These results reveal a fundamental role of buffering in biological regulation: enabling the simultaneous, independent control of multiple coupled outputs, which adds to its known role of regulating 'fast' disturbances and stabilising feedback. These results also reveal that different buffers carry out different roles - in the phosphagen system, AMP deaminase and adenylate kinase carry out the former role, while creatine phosphate and adenylate kinase (to a much lesser extent) carry out the latter roles.

Author Contributions

Conceptualization, E.J.H., and J.A.; Methodology, E.J.H., and J.A.; Formal Analysis: E.J.H., and J.A.; Investigation, E.J.H., and J.K.; Writing - Original Draft, E.J.H.; Writing - Review & Editing, E.J.H., J.A., and J.K.; Funding Acquisition, E.J.H.; Supervision, E.J.H. and J.A.

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None of the authors of this manuscript have a financial interest related to this work.

Appendix

Figures

Data sources for Figures 4 and 6 are A - [30], B-[19], C - [10], D - [22], E-[13], F- [21]. For repeated cases, the first case shows changes during ATP depletion and the second case shows changes during ATP regeneration.

In Figure 5, (b) shows sensitivity with $K_a = 1$ and (c) shows sensitivity with $K_a = 1$ and $B_a = 1$.

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Supplementary Information

SI1 Controllability Analysis

In this section, we study the controllability of ATP, ADP and AMP with buffering and feedback. Controllability is an important system property that determines whether overall regulation and control is possible (see [36, 4] for controllability background). Controllability determines the ability for systems inputs (e.g. enzyme activity) to be able to change the states (e.g. metabolite concentrations) of a system.

To model regulatory mechanisms, we assume that the reaction AMP→ADP occurs via adenylate kinase and that regeneration of ATP, ADP and AMP from other sources occurs via the regeneration of AMP from IMP. Other regeneration reactions also occur in cells, such as the production of AMP from the degradation of RNA, where these reactions are incorporated in the models as disturbances rather than energy regulation mechanisms. We do not make assumptions regarding ATP consumption and the models incorporate all forms of these processes. In the main section and below, we also discuss the case incorporating energy buffers that are closely related to the phosphagen system, showing equivalent results.

For clarity of results, we also assume that the reactions all occur in a single compartment. Without this assumption, we would need to model the phosphagen system and corresponding adenine nucleotide concentrations in multiple compartments, which would unnecessarily complicate analysis without providing greater insight.

Consider the general input-output model of ATP and ADP

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_e(ATP, ADP, z, u_h) + u_b + d_a \\
\frac{d}{dt}[ADP] &= -f_e(ATP, ADP, z, u_h) - nu_b + d_b \\
\frac{d}{dt}z &= f_m(ATP, ADP, z, u_h) + d_z
\end{aligned} \tag{S1}$$

where z is an m dimensional state representing metabolites excluding ATP and ADP, f_e represents the net flux of all ATP production and removal, f_m is the net flux of all z production and removal, u_h is a p dimensional input represent the input for feedback control on ATP production, u_b represent the buffering input or buffering flux, and n represents the stoichiometry of the buffering reaction ($n = 2$ for AK and $n = 1$ for pCr). d_a, d_b, and d_z represent disturbances to the process. We ignore $ATP \to AMP$ and $ADP \to AMP$ reactions as they would not be regenerated without adenylate kinase or a similar mechanism. This would result in a stable equilibrium at $ATP = 0$, and so is not useful or informative for controllability analysis.

The steady state $([ATP]_{ss}, [ADP]_{ss}, \bar{z})$ of (S3) occurs when $f_e = 0$, $f_m = 0$ and $u_b = 0$ for $n \neq 1$ and occurs when $f_e + u_b = 0$ and $f_m = 0$ for $n = 1$. In the following, we use the notation $\bar{u}_b$ for the steady state of the buffer input.

To simplify controllability analysis, we transform the model, such that

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_1(ATP, D, z, u_h) + u_b + d_a \\
\frac{d}{dt}z &= f_2(ATP, D, z, u_h) + d_z \\
\frac{d}{dt}D &= -(n-1)u_b + d_a + d_b
\end{aligned} \tag{S2}$$

where $D = [ATP] + [ADP]$,

$$\begin{aligned}
f_1(ATP, D, A, z, u_h) &= f_e(ATP, D - ATP, z, u_h) \\
f_2(ATP, D, A, z, u_h) &= f_m(ATP, D - ATP, z, u_h).
\end{aligned}$$

For controllability, we study the nominal case where disturbances are zero ($d_a = d_b = d_c = d_z = 0$).

We can observe in Equation (S4) that D is only controllable with u_b and with $n \neq 1$. Without u_b or with $n = 1$ then D is constant. Below shows the same results with a more formal linear controllability analysis.

We use a linearisation of the model for analysis, which is consistent with the linearized approach in all other sections of the paper. Linearising (S4) and placing in a standard control form, we have

$$\begin{aligned}
\Delta\dot{x} &= A\Delta x + B\Delta u \\
\Delta x &= \begin{bmatrix} \Delta ATP \\ \Delta z \\ \Delta D \end{bmatrix}, \quad \Delta u = \begin{bmatrix} \Delta u_h \\ \Delta u_b \end{bmatrix} \\
A &= \begin{bmatrix} f_{1a} & f_{1z} & f_{1D} \\ f_{2a} & f_{2z} & f_{2D} \\ 0 & 0 & 0 \end{bmatrix}, \quad B = \begin{bmatrix} f_{1u} & 1 \\ f_{2u} & 0 \\ 0 & (n-1) \end{bmatrix}
\end{aligned}$$

where $\Delta ATP = [ATP] - [ATP]_{ss}$, $\Delta D = D - \bar{D}$, $\bar{D} = [ATP]_{ss} + [ADP]_{ss}$, $\Delta z = z - \bar{z}$, $\Delta u_h = u_h - \bar{u_h}$ and $\Delta u_b = u_b - \bar{u_b}$ are the deviations of the respective variables from their steady states, and

$$f_{ia} = \frac{\partial f_i}{\partial [ATP]}, \quad f_{iz} = \frac{\partial f_i}{\partial z}, \quad f_{iD} = \frac{\partial f_i}{\partial D}, \quad f_{iu} = \frac{\partial f_i}{\partial u_h}$$

are the linearisations of the functions about the steady states, where f_i represents f_1 or f_2.

The controllability matrix is $\zeta = [B, AB, A^2B, \ldots, A^{m+2}B]$ and the system is controllable if the matrix has a rank of $m + 2$ [36, 4].

We first note that if there is no buffer control then

$$B = \begin{bmatrix} f_{1u} \\ f_{2u} \\ 0 \end{bmatrix}, \quad \zeta = \begin{bmatrix} f_{1u} & f_{1a}f_{1u} + f_{1z}f_{2u} & \ldots & \\ f_{2u} & f_{2a}f_{1u} + f_{2z}f_{2u} & \ldots & \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

and so the system is not controllable as the matrix rank is less than $m + 2$.

If there is buffer control then

$$\zeta = \begin{bmatrix} f_{1u} & 1 & f_{1a}f_{1u} + f_{1z}f_{2u} & f_{1a} + (n-1)f_{1D} & \ldots \\ f_{2u} & 0 & f_{2a}f_{1u} + f_{2z}f_{2u} & 0 & \ldots \\ 0 & (n-1) & 0 & 0 & \ldots \end{bmatrix}$$

If we assume that the subsystem that excludes D is controllable then the top $m + 1$ rows of ζ have a rank of $m + 1$. In this case, the matrix has rank $m + 2$ if $n \neq 1$, and so the system is controllable for AK ($n = 2$) but not pCr ($n = 1$).

We can also consider the case where the assumptions do not hold and reaction AMP→ADP occurs via reaction other than adenylate kinase e.g. specific forms of polyphosphate kinase [1], where polyphosphate carries out an analogous role to creatine phosphate in microorganisms. Consider the general input-output model of ATP and ADP

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_e(ATP, ADP, z, u_h) + u_b + d_a \\
\frac{d}{dt}[ADP] &= -f_e(ATP, ADP, z, u_h) - nu_b + u_p + d_b \\
\frac{d}{dt}z &= f_m(ATP, ADP, z, u_h) + d_z
\end{aligned} \tag{S3}$$

where u_p represents enzymes that can carry out AMP→ADP. To simplify controllability analysis, we once again transform the model, such that

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_1(ATP, D, z, u_h) + u_b + d_e + d_a \\
\frac{d}{dt}z &= f_2(ATP, D, z, u_h) + d_z \\
\frac{d}{dt}D &= -(n-1)u_b + u_p + d_a + d_f
\end{aligned} \tag{S4}$$

where $D = [ATP] + [ADP]$,

$$\begin{aligned}
f_1(ATP, D, A, z, u_h) &= f_e(ATP, D - ATP, z, u_h) \\
f_2(ATP, D, A, z, u_h) &= f_m(ATP, D - ATP, z, u_h).
\end{aligned}$$

Again, for controllability, we study the nominal case where disturbances are zero ($d_a = d_b = d_c = d_z = 0$).

We can observe in Equation (S4) that D is only controllable with either u_p or u_b. Without either mechanism then D is constant. Formal linear controllability analysis can be completed as above with the same results.

We next look at the case for AMP deaminase. Consider the general input-output model of ATP, ADP and AMP

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_e(ATP, ADP, AMP, z, u_h, u_b) - f_p(ATP, ADP, AMP, z, u_h, u_b) + d_a \\
\frac{d}{dt}[ADP] &= -f_e(ATP, ADP, AMP, z, u_h, u_b) + f_b(ATP, ADP, AMP, z, u_h, u_b) + d_b \\
\frac{d}{dt}[AMP] &= f_p(ATP, ADP, AMP, z, u_h) - f_b(ATP, ADP, AMP, z, u_h, u_b) + u_r + d_c \\
\frac{d}{dt}z &= f_m(ATP, ADP, AMP, z, u_h) + d_z
\end{aligned} \tag{S5}$$

where z is an m dimensional state representing metabolites excluding ATP, ADP and AMP, u_b represent the buffering input (e.g. Adenylate Kinase) and u_r represents the buffering input from AMP deaminase. f_e represents the net flux of all $ATP \leftrightarrow ADP$, f_p represents the net flux of all $ATP \leftrightarrow AMP$ and f_b represents the net flux of all $ADP \leftrightarrow AMP$. d_a, d_b, d_c and d_z represent disturbances to the process.

The steady state $([ATP]_{ss}, [ADP]_{ss}, [AMP]_{ss}, \bar{z})$ of (S5) occurs when $f_m = 0$, $u_r = 0$, $f_e = f_b = f_p$. In the following, we use the notation $\bar{u}_r$ for the steady state of the AMPD input.

To simplify controllability analysis, we transform the model, such that

$$\begin{aligned}
\frac{d}{dt}[ATP] &= f_1(ATP, D, A, z, u_h, u_b) + d_a \\
\frac{d}{dt}z &= f_2(ATP, D, A, z, u_h, u_b) + d_z \\
\frac{d}{dt}D &= f_3(ATP, D, A, z, u_h, u_b) + d_a + d_b \\
\frac{d}{dt}A &= u_r + d_a + d_b + d_c
\end{aligned} \tag{S6}$$

where $D = [ATP] + [ADP]$, $A = [ATP] + [ADP] + [AMP]$, and

$$\begin{aligned}
f_1(ATP, D, A, z, u_h) &= f_e(ATP, D - ATP, A - D, z, u_h, u_b) - f_p(ATP, D - ATP, A - D, z, u_h, u_b) \\
f_2(ATP, D, A, z, u_h) &= f_m(ATP, D - ATP, A - D, z, u_h, u_b) - f_b(ATP, D - ATP, A - D, z, u_h, u_b) \\
f_3(ATP, D, A, z, u_h) &= f_b(ATP, D - ATP, A - D, z, u_h, u_b) - f_p(ATP, D - ATP, A - D, z, u_h, u_b).
\end{aligned}$$

For controllability, we again study the nominal case where disturbances are zero ($d_a = d_b = d_c = d_z = 0$).

We can observe in (S6) that A is only controllable with u_r. Without u_r then A is constant. Below, we carry out a more formal linear controllability analysis.

Linearising (S6) and placing in a standard control form, we have

$$\Delta\dot{x} = A\Delta x + B\Delta u$$
$$\Delta x = \begin{bmatrix} \Delta ATP \\ \Delta z \\ \Delta D \\ \Delta A \end{bmatrix}, \quad \Delta u = \begin{bmatrix} \Delta u_h \\ \Delta u_b \\ \Delta u_r \end{bmatrix}$$
$$A = \begin{bmatrix} f_{1a} & f_{1z} & f_{1D} & f_{1A} \\ f_{2a} & f_{2z} & f_{2D} & f_{2A} \\ f_{3a} & f_{3z} & f_{3D} & f_{3A} \\ 0 & 0 & 0 & 0 \end{bmatrix}, \quad B = \begin{bmatrix} f_{1u} & f_{1b} & 0 \\ f_{2u} & f_{1b} & 0 \\ f_{3u} & f_{1b} & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

where $\Delta ATP = [ATP] - [ATP]_{ss}$, $\Delta D = D - \bar{D}$, $\bar{D} = [ATP]_{ss} + [ADP]_{ss}$, $\Delta A = A - \bar{A}$, $\bar{A} = [ATP]_{ss} + [ADP]_{ss} + [AMP]_{ss}$, $\Delta z = z - \bar{z}$, $\Delta u_h = u_h - \bar{u_h}$, $\Delta u_b = u_b - \bar{u_b}$, $\Delta u_r = u_r - \bar{u_r}$ are the deviations of the respective variables from their steady states, and

$$f_{ia} = \frac{\partial f_i}{\partial [ATP]}, \quad f_{iz} = \frac{\partial f_i}{\partial z}, \quad f_{iD} = \frac{\partial f_i}{\partial D}, \quad f_{iA} = \frac{\partial f_i}{\partial A}, \quad f_{iu} = \frac{\partial f_i}{\partial u_h}, \quad f_{ib} = \frac{\partial f_i}{\partial u_b}$$

are the linearisations of the functions about their steady states, where f_i represents f_1, f_2 or f_3.

The controllability matrix is $\zeta = [B, AB, A^2B, \ldots, A^{m+2}B]$ and the system is controllable if the matrix has a rank of $m + 3$ [36, 4].

We first note that if there is no AMP deaminase control ($u_r = 0$) then

$$B = \begin{bmatrix} f_{1u} \\ f_{2u} \\ f_{3u} \\ 0 \end{bmatrix}, \quad \zeta = \begin{bmatrix} f_{1u} & f_{1b} & f_{1a}f_{1u} + f_{1z}f_{2u} + f_{1D}f_{3u} & \cdots \\ f_{2u} & f_{2b} & f_{2a}f_{1u} + f_{2z}f_{2u} + f_{2D}f_{3u} & \cdots \\ f_{3u} & f_{3b} & f_{3a}f_{1u} + f_{3z}f_{2u} + f_{3D}f_{3u} & \cdots \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

and so the system is not controllable as the matrix rank is less than $m + 3$.

If there is AMP deaminase control u_r then

$$\zeta = \begin{bmatrix} f_{1u} & f_{1b} & 0 & f_{1a}f_{1u} + f_{1z}f_{2u} + f_{1D}f_{3u} & \cdots \\ f_{2u} & f_{2b} & 0 & f_{2a}f_{1u} + f_{2z}f_{2u} + f_{2D}f_{3u} & \cdots \\ f_{3u} & f_{3b} & 0 & f_{3a}f_{1u} + f_{3z}f_{2u} + f_{3D}f_{3u} & \cdots \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix}$$

Thus if we assume that the subsystem without A and u_r is controllable (first $m + 2$ rows has a rank of $m + 2$) then the full system with AMP deaminase (u_r) is controllable.

Thus the energy metabolism system is only controllable with both AK and AMP deaminase. Further, without AK and AMPD then feedback on metabolic pathways is fundamentally limited to only controlling one combination of ATP, ADP and AMP.

SI2 Buffer Analysis Background

This section of the Appendix provides background on the buffer equilibrium ratio and sensitivity function as well as their importance in studying temporal dynamics and differential equation models of regulation.

SI2.1 General Buffering Models, Linearization and the Buffer Equilibrium Ratio

Consider the model

$$\begin{aligned}
\dot{y} &= -\underbrace{g_y(y,x) + g_x(x,y)}_{\text{buffering}} + \underbrace{p_y(y,z)}_{\substack{\text{production}\\ \text{with feedback}}} - \underbrace{\nu_y(y,z)}_{\text{removal}} + \underbrace{d_y(t)}_{\text{disturbance}} \\
\dot{x} &= \underbrace{g_y(z,x) - g_x(x,z)}_{\text{buffering}} - \underbrace{\nu_x(x)}_{\text{removal}} \\
\dot{z} &= \underbrace{p_z(y,z)}_{\substack{\text{production}\\ \text{with feedback}}} - \underbrace{\nu_z(y,z)}_{\text{removal}}
\end{aligned} \tag{S7}$$

where y is the regulated output, x is the buffering species, and z represents the other system concentrations, p_y, p_z are the production rate of y and z, ν_y, ν_z and ν_x are the removal rates, the (lumped) buffering reactions are $g_y(y,x)$ representing $y \to x$ and $g_x(x,y)$ representing $x \to x$, and d_y is a disturbance. Incorporation of feedback into this class of systems is represented by the y dependence of the production terms p_y, p_z. We define the nominal steady state of the system to be its zero-disturbance ($d_y = 0$) steady state, and denote it as $\bar{y}$, with a corresponding states $\bar{z}$ and $\bar{x}$; here y is called the set point of the regulated variable.

The nominal steady state $(\bar{y}, \bar{x}, \bar{z})$ of Equation (S7) occurs when

$$\begin{aligned}
g_y(\bar{y},\bar{x}) &= g_x(\bar{x},\bar{y}) + \nu_x(\bar{x}) \quad \text{(buffering rates at steady state)} \\
p_z(\bar{y},\bar{z}) &= \nu_z(\bar{y},\bar{z}) \quad \text{(production/removal rates at steady state).} \\
p_y(\bar{y},\bar{z}) &= \nu_y(\bar{y},\bar{z}) + \nu_x(\bar{x}) \quad \text{(production/removal rates at steady state).}
\end{aligned}$$

To analyze the model, we reformulate (S7) in terms of deviations $\Delta y = y - \bar{y}$, $\Delta x = x - \bar{x}$, $\Delta z = z - \bar{z}$ and linearize about the nominal steady state to obtain

$$\Delta\dot{y} = -b_y\Delta y + b_x\Delta x - h_{yy}\Delta y - h_{yz}\Delta z - \gamma_{yy}\Delta y - \gamma_{yz}\Delta z + d_y(t) \tag{S8}$$

$$\Delta\dot{x} = b_y\Delta z - b_x\Delta x - \gamma_x\Delta x \tag{S9}$$

$$\Delta\dot{z} = -h_{zy}\Delta y - h_{zz}\Delta z - \gamma_{zy}\Delta y - \gamma_{zz}\Delta z \tag{S10}$$

$$\tag{S11}$$

where

$$\begin{aligned}
h_{yy} &= -\frac{\partial}{\partial y} p_y(y,z)|_{(y,z)=(\bar{y},\bar{z})}, & h_{yz} &= -\frac{\partial}{\partial z} p_y(y,z)|_{(y,z)=(\bar{y},\bar{z})}, \\
h_{zy} &= -\frac{\partial}{\partial y} p_z(y,z)|_{(y,z)=(\bar{y},\bar{z})}, & h_{zz} &= -\frac{\partial}{\partial y} p_z(y,z)|_{(y,z)=(\bar{y},\bar{z})}, \\
b_y &= \frac{\partial}{\partial y}(g_y(y,x) - g_x(x,y))|_{(y,x)=(\bar{y},\bar{x})}, & b_x &= \frac{\partial}{\partial x}(g_x(x,y) - g_y(y,x))|_{(y,x)=(\bar{y},\bar{x})}, \\
\gamma_{yy} &= \frac{d\nu_y}{dy}(y,z)|_{(y,z)=(\bar{y},\bar{z})}, & \gamma_{yz} &= \frac{d\nu_y}{dz}(y,z)|_{(y,z)=(\bar{y},\bar{z})}, \\
\gamma_{zy} &= \frac{d\nu_z}{dy}(y,z)|_{(y,z)=(\bar{y},\bar{z})}, & \gamma_{zz} &= \frac{d\nu_z}{dz}(y,z)|_{(y,z)=(\bar{y},\bar{z})}, \\
\gamma_x &= \frac{d\nu_x}{dx}(x)|_{x=\bar{x}}.
\end{aligned}$$

In the main section, we define the buffer equilibrium ratio to be

$$B = \frac{\Delta x}{\Delta y} \tag{S12}$$

when the buffering reactions are at (quasi)-equilibrium.

In model (S8), if we assume (rapid) equilibrium buffering [17] with quasi-equilibrium of Δy and Δx (by setting $\Delta\dot{x} = 0$) and slow variable $\Delta y_T = \Delta y + \Delta x$, then we obtain

$$\begin{aligned} &\Delta x = B\Delta y, \quad B = \frac{b_y}{b_x + \gamma_x} \\ &\Delta\dot{y}_T = -h_{yy}\Delta y - h_{yz}\Delta z - \gamma_{yy}\Delta y - \gamma_{yz}\Delta z - \gamma_x\Delta x + d_y(t) \end{aligned} \tag{S13}$$

where we can see that (S12) occurs naturally.

Substituting $\Delta x = B\Delta y$ into $\Delta y_T = \Delta y + \Delta x$ we have

$$\Delta y_T = (1 + B)\Delta y \tag{S14}$$

and so

$$\begin{aligned} (1 + B)\Delta\dot{y} &= -h_{yy}\Delta y - h_{yz}\Delta z - \gamma_{yy}\Delta y - \gamma_{yz}\Delta z - B\gamma_x\Delta y + d_y(t) \\ \Delta\dot{z} &= -h_{zy}\Delta y - h_{zz}\Delta z - \gamma_{zy}\Delta y - \gamma_{zz}\Delta z \end{aligned} \tag{S15}$$

where p_Δ and ν_Δ represent the linearized production and removal terms. The y component of Equation (S15) can be rearranged in the form

$$(1 + B)\Delta\dot{y} = \underbrace{p_\Delta - \nu_\Delta}_{\substack{\text{linearized} \\ \text{production} \\ \text{\& removal}}} + d_y \tag{S16}$$

where it can be observed that B, which is typically positive, reduces the rate of change on the LHS.

SI2.2 Calculating Buffer Equilibrium Ratio B

We can use an explicit or implicit approach to calculate the buffer equilibrium ratio. If we have an explicit function of the buffer concentration $x = x(y)$ in terms of output y (e.g. assuming buffering at quasi-equilibrium), then we can calculate buffer equilibrium ratio B directly using

$$B = \frac{dx}{dy}(y)\Big|_{y=\bar{y}}. \tag{S17}$$

We can also have an implicit relationship between x and y by setting $\dot{x} = 0$ in (S7), where

$$g_y(y, x) = g_x(x, y) + \nu_x(x). \tag{S18}$$

We can use the implicit function theorem, which results in

$$\begin{aligned} &B = \frac{d\bar{x}}{d\bar{y}}(y) = -\frac{\frac{\partial g_{net}}{\partial y}}{\frac{\partial g_{net}}{\partial x} + \frac{\partial \nu_x}{\partial x}} = \frac{b_y}{b_x + \gamma_x} \\ &g_{net}(y, x) = g_y(y, x) - g_x(x, y) \end{aligned} \tag{S19}$$

where b_y, b_x and γ_x are defined under (S8). For this case with no removal process ($\gamma_x = 0$) then

$$B = \frac{\Delta x}{\Delta y} = \frac{b_y}{b_x}. \tag{S20}$$

SI2.3 Equivalence of Buffers to Feedback

PID (Proportional-Integral-Derivative) feedback controllers are highly important in control theory and are ubiqitous regulatory components of many technological systems [4]. Proportional and integral feedback have been well studied in biological contexts [28, 31, 12, 24, 2, 23, 26, 27, 3, 8]. However, significantly less is understood about the role of derivative feedback. Our recent work showed that rapid buffering is equivalent to derivative feedback and that the buffer equilibrium ratio represents the magnitude of "derivative feedback" [17, 16]. This can be seen by noting that with rapid buffering (S16) can be rearranged as

$$\Delta\dot{y} = \underbrace{-B\Delta\dot{y}}_{\substack{\text{derivative}\\ \text{feedback}}} + \underbrace{p_\Delta - \nu_\Delta}_{\substack{\text{linearized}\\ \text{production}\\ \text{\& removal}}} + d_y. \tag{S21}$$

SI2.4 Example of a Sensitivity Function

We next calculate the sensitivity function for a simple example with an oscillating disturbances. The sensitivity function is (see Section 2)

$$\phi = \frac{\|\Delta y\|_t}{\bar{y}} \Big/ \frac{\|d_y\|_t}{\bar{p}} \tag{S22}$$

where $\|\Delta y\|_t$ represents the amplitude of oscillations of the output, $\|\Delta d_y\|_t$ represents the amplitude of oscillations of the disturbance, the output oscillations are normalised by the output set point and the disturbance oscillations are normalised by the steady state production.

In the following example we look at the simplest case of (S7), where the only species are a buffer and a regulated species. In this case, there are no z variables in (S13), and for simplicity we also assume that $\nu_x = 0$, such that (S7) becomes

$$\begin{aligned} \dot{y} &= -g_y(y,x) + g_x(x,y) + p_y(y,z) - \nu_y(y)y + d_y(t) \\ \dot{x} &= g_y(z,x) - g_x(x,z) \end{aligned} \tag{S23}$$

where the steady state occurs when $p_y(\bar{y}) = \nu_y(\bar{y})$ and $g_y = g_x$. For this case, the linearisation (S16) becomes

$$(1+B)\Delta\dot{y} = -h_y\Delta y - \gamma_y\Delta y + d_y\ , \tag{S24}$$

where we replace h_{yy} by h_y and γ_{yy} by γ_y. Normalising time in (S15) by the unregulated time-scale $1/\gamma_y$, we have

$$(1+B)\gamma_y\Delta\dot{y} = -h_y\Delta y - \gamma_y\Delta y - B\gamma_x\Delta y + d_y\ , \tag{S25}$$

We can use Fourier transforms to determine the relative size and phase of oscillating outputs against oscillating disturbances [4]. From (S22), the sensitivity function is

$$\phi_\omega = \frac{\|\Delta y\|_t}{\bar{y}} \Big/ \frac{\|d_y\|_t}{\bar{p}} = \frac{\bar{p}}{\bar{y}} \left| \frac{Y(i\omega)}{D_y(i\omega)} \right| \tag{S26}$$

where Y is the Fourier transform of y, D_y is the Fourier transform of d_y, ω_n is the frequency of the oscillations, and $|\cdot|$ is the magnitude of the transform. The transfer function for (S25) is

$$\begin{aligned} G(i\omega_n) = \frac{Y(i\omega_n)}{D_y(i\omega_n)} &= \frac{1}{(1+B)\gamma_y i\omega_n + \gamma_y + h_y} \\ &= \frac{1}{(1+B)\gamma_y i\omega_n + \gamma_y\,(1+H)} \end{aligned}$$

where we set

$$H = \frac{\bar{y}}{\bar{p}} h_y.$$

Taking the magnitude of the transfer functions, we have

$$|G(i\omega_n)|^2 = \frac{|Y(i\omega_n)|^2}{|D_y(i\omega_n)|^2} = \frac{1}{\gamma_y^2} \frac{1}{(1+B)^2\omega_n^2 + (1+H)^2}.$$

Substituting into (S26) and using $\bar{p} = \nu_y(\bar{y})$, we have

$$\begin{aligned} \phi_\omega^2 &= \alpha^2 \frac{1}{(1+B)^2\omega_n^2 + (1+H)^2} \\ &= \alpha^2 \frac{1}{(1+B)^2\omega_n^2 + (1+H)^2} \end{aligned}$$

where $\alpha = \nu_y(\bar{y})/(\gamma_y \bar{y})$ is a measure of the nonlinearity of the removal of y [17]. If the removal of y is linear then $\nu_y(\bar{y}) = \gamma_y \bar{y}$ and $\alpha = 1$. We can observe that the sensitivity function is a function of ω_n, H and B and α. For the case of no feedback, we have

$$\phi_\omega^2 = \alpha^2 \frac{1}{(1+B)^2\omega_n^2 + 1}.$$

SI3 pH Buffering in Open Systems

The reader may find it instructive to compare and contrast the open-system metrics introduced in Section 2 to familiar metrics commonly used to analyze closed-system buffering. For ease of readability, we describe the results in SI3 and include the working in SI4.

Consider the well-studied application of pH buffering, and more specifically, the simple example of a weak acid buffer. Such a system can be represented by the reaction

$$\mathrm{H}^+ + \mathrm{A}^- \leftrightarrow \mathrm{HA} \tag{S27}$$

where HA is a weak acid that buffers H^+ (and thus pH, given that $\mathrm{pH} = -\log_{10}[\mathrm{H}^+]$), and the thermodynamic equilibrium constant $K = [\mathrm{H}^+][\mathrm{A}^-]/[\mathrm{HA}]$. (Here, we ignore the water ionization reaction.) For reaction (S27), the buffer equilibrium ratio B is (see SI4.1)

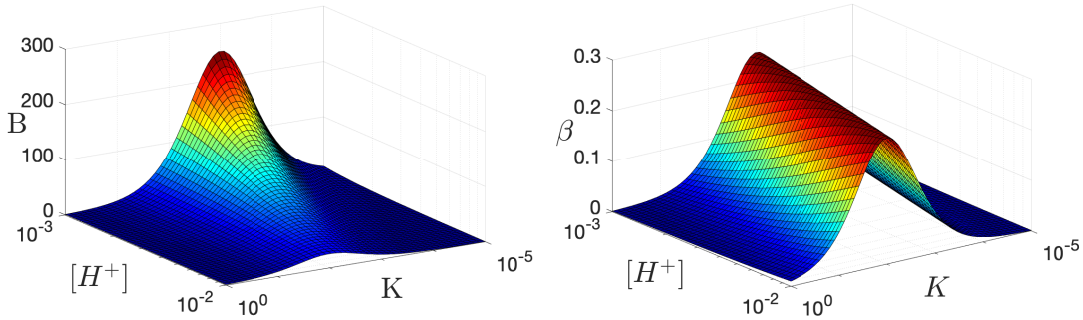
$$B = \frac{\Delta x}{\Delta y} = \frac{\Delta[\mathrm{HA}]}{\Delta[\mathrm{H}^+]} = \frac{CK}{(K + [\mathrm{H}^+])^2} \tag{S28}$$

where the buffer concentration is $x = [\mathrm{HA}]$, the output concentration is $y = [\mathrm{H}^+]$, $C = [\mathrm{HA}] + [\mathrm{A}^-]$, and $\bar{y} = [\mathrm{H}^+]$ is at the output set point. Figure S1 demonstrates that B is a function of $[\mathrm{H}^+]$ and K, increasing with decreasing $[\mathrm{H}^+]$. If $[\mathrm{H}^+]$ is fixed, B takes on a maximum when $K = [\mathrm{H}^+]$.

In pH theory, the buffer capacity metric, $\beta = \Delta n/\Delta\mathrm{pH}$, is a measure of the resistance of a buffer solution to pH change on addition of a strong base (n represents the moles of added OH^- ions) [37]. The sensitivity function, ϕ, is its (inverted) open-system generalization [37]:

$$\beta^{-1} = \frac{\Delta\mathrm{pH}}{\Delta n} = \frac{1}{2.3} \frac{\Delta[\mathrm{H}^+]}{[\mathrm{H}^+]} \Big/ \Delta n \tag{S29}$$

where $[\mathrm{H}^+] = y$ is the output signal being regulated. The form of (S29) is similar to (5) with a few exceptions: (a) the 2.3 factor, which results from the $\log(10)$ scaling of pH; (b) the disturbance, $\Delta[\mathrm{H}^+]$



(a) pH Buffer Equilibrium Ratio

(b) pH Buffer Capacity

Figure S1: pH buffer capacity (bottom) and buffer equilibrium ratio (top) with $C = [A^-] + [HA] = 1$. β has concentration units while B is dimensionless.

can be interpreted as a one-time addition of mass (OH^-) to an otherwise closed system, whereas the sensitivity function, ϕ, considers a system where the disturbance, d_y, is directly on the rate of change of the output signal; and (c) the disturbance, $\Delta[H^+]$, is not normalized by a production term, as there is no on-going rate of H^+ production in the closed system.

Note that in the closed pH buffering system, both B and β convey the isolated effectiveness of the buffer. In fact,

$$\begin{aligned}\beta &= 2.3[H^+] + 2.3\frac{CK[H^+]}{(K+[H^+])^2} \\ &= 2.3[H^+](1+B)\end{aligned} \tag{S30}$$

(Furthermore, the constant $2.3[H^+]$ term is often ignored, as it typically achieves significance at pH levels well below levels of interest [37].) In the open systems, whilst B still represents an isolate measure of the buffer, the sensitivity function, ϕ, depends upon the type of disturbance and other forms of regulation present [16, 17] (see SI2.4 for a simple example).

The plot of the buffer capacity β can be seen in Figure S1. If $[H^+]$ is fixed, then buffer capacity β is maximum when $K = [H^+]$, similar to B. However, if $[H^+]$ is varied then β is maximum when $[H^+] = K$, rather than a maximum of B at small $[H^+]$. This change results from the buffer capacity including an extra $[H^+]$ for normalisation, which in open systems is included in the sensitivity function (5) rather than B.

SI4 Buffer Equilibrium Ratios

This section of the Appendix provides supporting evidence of buffer equilibrium ratio calculations presented in the main section of the paper. We determine the buffer equilibrium ratios for different reactions.

SI4.1 pH Buffering

The first reaction that we study is the pH buffering reaction

$$H^+ + A^- \leftrightarrow HA$$

which can be written as

$$y + z_c \underset{k_x}{\overset{k_y}{\rightleftharpoons}} x$$

where $y = [H^+]$, $x = [HA]$ and cosubtrate $z_c = [A^-]$. The forward and reverse reaction rates are

$$g_y = k_y z_c y, \quad g_x = k_x x$$

and at equilibrium we have

$$K = \frac{k_x}{k_y} = \frac{yz}{x}. \tag{S31}$$

Setting $C = z_c + x$ and rearranging, we have

$$x = \frac{Cy}{K + y}.$$

The buffer equilibrium ratio can be calculated as

$$B = \frac{dx}{dy}\Big|_{y=\bar{y}} = \frac{CK}{(K + \bar{y})^2}.$$

Substituting $\bar{y} = [H^+]$ then buffer equilibrium ratio is

$$\boxed{B = \frac{\Delta x}{\Delta y} = \frac{\Delta[HA]}{\Delta[H^+]} = \frac{CK}{(K + [H^+])^2}} \tag{S32}$$

which is shown in (S27) in SI3.

SI4.1.1 Optimal Parameters

For fixed kinetic constants, B is maximum when $\bar{y} = 0$, for which $B = C/K$. B approaches zero when $\bar{y} \to \infty$. The buffer equilibrium ratio B can also be increased linearly by increasing C.

For a fixed non-zero $\bar{y}$, the maximum buffer equilibrium ratio occurs when

$$\begin{aligned} \frac{\partial B}{\partial K} &= C\frac{(K + \bar{y})^2 - 2K(K + \bar{y})}{(K + \bar{y})^4} \\ &= C\frac{\bar{y} - K}{(K + \bar{y})^3} = 0 \end{aligned}$$

which occurs when

$$\bar{y} = K$$

and results in

$$B = \frac{C}{4\bar{y}}.$$

For a fixed non-zero K, the maximum buffer equilibrium ratio occurs when $\bar{y} = 0$, and results in

$$B = \frac{C}{K}.$$

SI4.2 Creatine Phosphate

The next reaction that we study is the creatine kinase reaction

$$ATP + Cr \leftrightarrow pCr + ADP$$

where Cr is creatine and pCr is the creatine phosphate that buffers ATP. We can rewrite this in the form

$$y + z_c \underset{k_x}{\overset{k_y}{\rightleftharpoons}} x_c + z_a$$

where $z_c = [Cr]$, $x = [pCr]$, $y = [ATP]$ and $z_a = [ADP]$.

SI4.2.1 Buffer Equilibrium Ratio: Constant Parameters

The forward and reverse reaction rates are

$$g_y = k_y z_c y, \quad g_x = k_x z_a x_c.$$

and at equilibrium we have

$$K_c = k_x/k_y = \frac{y z_c}{x_c z_a}. \tag{S33}$$

Setting $C = z_c + x$ and $D = y + z_a$ and rearranging, we have

$$x_c = \frac{Cy}{K_c D + (1 - K_c) y} \tag{S34}$$

The Buffer Equilibrium Ratio for fixed parameters is

$$\begin{aligned} B_c^f = \left.\frac{dx_c}{dy}\right|_{y=\bar{y}} &= \frac{CDK_c}{(K_c D + (1-K_c)\bar{y})^2} \\ &= \frac{C}{D}\frac{K_c}{\left(K_c + (1-K_c)\frac{\bar{y}}{D}\right)^2} \end{aligned}$$

which is a function of the three independent inputs K_c, $\bar{y}/D$ and C/D. Substituting $\bar{y} = [ATP]$, we have

$$\boxed{B_c^f = \frac{C}{D}\frac{K_c}{\left(K_c + (1-K_c)\frac{ATP}{D}\right)^2}} \tag{S35}$$

which is shown in (1) in the main section.

SI4.2.2 Buffer Equilibrium Ratio: Varying parameters K_c and $A = ATP + ADP$

If K and D in (S34) vary with ATP then we need to incorporate varying parameters into the buffer equilibrium ratio B_c. From (S34), we have the buffer concentration

$$x_c = \frac{Cy}{KD + (1-K)y}$$

and so

$$B_c = \left.\frac{dx_c}{dy}\right|_{y=\bar{y}} = \frac{CDK}{(KD + (1-K)\bar{y})^2} - \frac{C\bar{y}(D-\bar{y})}{(KD + (1-K)\bar{y})^2}\frac{dK}{dy} - \frac{C\bar{y}K}{(KD + (1-K)\bar{y})^2}\frac{dD}{dy}$$

where the derivatives of K and D are evaluated at $y = \bar{y}$. Using the notation $B_a = \frac{dD}{dy}$ (see Section SI4.3 for further explanation) then

$$B_c = \frac{CDK}{(KD + (1-K)\bar{y})^2} - \frac{C\bar{y}(D-\bar{y})}{(KD + (1-K)\bar{y})^2}\frac{dK}{dy} - \frac{C\bar{y}K}{(KD + (1-K)\bar{y})^2}B_a.$$

Using (S35), we set

$$B_c^f = \frac{CDK}{(KD + (1-K)\bar{y})^2}$$

and so

$$B_c = B_c^f \left(1 - \left(1 - \frac{\bar{y}}{D}\right) \frac{\bar{y}}{K} \frac{dK}{dy} - \frac{\bar{y}}{D} B_a\right). \tag{S36}$$

Substituting $\bar{y} = [ATP]$, we have

$$\boxed{B_c = B_c^f \left(1 - \left[1 - \frac{ATP}{D}\right] \underbrace{\frac{ATP}{K_c} \frac{\Delta K_c}{\Delta ATP}}_{\text{K/pH}} - \underbrace{\frac{ATP}{D} B_a}_{\text{AK}}\right)} \tag{S37}$$

which is shown in (7) in the main section.

Equilibrium constant K_c typically increases with decreasing ATP (via decreasing pH), where $\Delta K_c / \Delta ATP < 0$ (see SI4.2.3). Thus the effect of changing K_c is to increase B_c in (7). In contrast, D decreases with decreasing ATP ($B_a > 0$) and so the effect in (7) of changing D is to reduce B_c. As such, adenylate kinase reduces the effectiveness of buffering by creatine phosphate in (7).

SI4.2.3 Comparison to Experimental Data

We next compare the theoretical and experimental values for B_c in human muscles and adypocyte. To determine the experimental B_c, observations of pCr and ATP over time (e.g. before and after a period of exercise) can be used in the formula

$$B_c = \frac{\Delta[pCr]}{\Delta[ATP]} = \frac{[pCr]_{final} - [pCr]_{initial}}{[ATP]_{final} - [ATP]_{initial}}. \tag{S38}$$

This formula is a simple method of calculation, but can result in larger measurement errors for large B if there are small changes to $[ATP]$.

To calculate the theoretical B over a range of y, we use

$$B_c = \frac{\Delta x}{\Delta y} = \frac{1}{\Delta y} \int_{y_0}^{y_1} B_c(y) dy \tag{S39}$$

where $\Delta y = y_1 - y_0$. We know experimental values of K_c, C/D and y/D for particular measurement points rather than all values, and so for fixed B we approximate the integral using the trapezoidal rule

$$B_c^f = \frac{1}{\Delta y} \sum_k \frac{1}{2} [B_c(y_{k+1}) + B_c(y_k)](y_{k+1} - y_k). \tag{S40}$$

The theoretical B_c^f is estimated from the experimental K, C and D at each data point.

We can use a similar approach for the case with varying parameters, where we average the fixed components and the K and D rate of change components. When applying the average for this case, we use the geometric average as the parameters can vary over orders of magnitude (see Table S1). We continue to use the arithmetic average for the fixed component for consistency with above. If we set

$$B_c = B_c^f + B_c^K + B_c^A, \quad B_c^K = -B_c \left(1 - \frac{y}{D}\right) \frac{y}{K} \frac{dK}{dy}, \quad B_c^A = -B_c \frac{y}{D} \frac{dD}{dy} \tag{S41}$$

then we use the approximation

$$\begin{aligned} B_c = & \frac{1}{\Delta y} \sum_k \frac{1}{2} [B_c(y_{k+1}) + B_c(y_k)](y_{k+1} - y_k) \\ & + \frac{1}{\Delta y} \sum_k S_K \sqrt{|B_c^K(y_{k+1}) B_c^K(y_k)|} (y_{k+1} - y_k) \\ & + \frac{1}{\Delta y} \sum_k S_A \sqrt{|B_c^A(y_{k+1}) B_c^A(y_k)|} (y_{k+1} - y_k) \end{aligned} \tag{S42}$$

where $S_K = \text{sign}(B_K(y_{k+1}) B_K(y_k))$ and $S_A = \text{sign}(B_A(y_{k+1}) B_A(y_k))$.

B_{exp}	B_{theor}	B_{theor} inc. $\frac{dK}{dy}, \frac{dD}{dy}$	K_{exp}	$\frac{ATP}{ATP+ADP}$ (exp)	Ref
11.5±8.6	5.8	12.6	4.9-190	0.88-0.83	[30](ATP ↓)
15.4±5.8	6.7	15.2	2.5-15.4	0.87-0.85	[19](ATP ↓)
20.9±9.9	7.1	19.0	15.4-2.6	0.85-0.87	[19] (ATP ↑)
7.5±2.2	5.5	8.4	17.9-138.8	0.87-0.82	[10](ATP ↓)
10.7±3.6	5.5	10.8	13.9-86.9	0.86-0.77	[10](ATP ↓)
7.3 ±1.5	7.7	8.2	5.8-76.5	0.90-0.79	[22](ATP ↓)
16.0±9.9	11.42	16.3	76.5-3.6	0.79-0.89	[22](ATP ↑)
8.3±1.5	8.3	7.6	4.6-42	0.92-0.87	[13] (ATP ↓)
36±49.5 *	9.6	35.6	42-5.4	0.87-0.89	[13] (ATP ↑)

Table S1: Buffer equilibrium ratio B_c of creatine phosphate for human muscle during and after exercise. *not shown in Figure 4 due to large experimental error.

SI4.3 Adenylate Kinase

We next study the reaction adenylate kinase

$$ATP + AMP \leftrightarrow 2ADP$$

that can be written in the general form

$$y + z_m \underset{k_x}{\overset{k_y}{\rightleftharpoons}} 2z_a.$$

We set

$$x_a = y + z_a = [ATP] = [ADP]$$

where the demand reaction $y \to z_d$ does not affect the buffer x_a for this buffer definition.

We note that

$$\frac{dx}{dt} = \frac{dy}{dt} + \frac{dz}{dt} = -g_y + g_x + 2g_y - 2g_x + ... = g_y - g_x + ...$$

and so the effect of the buffering reactions is equivalent to the buffering in the general model (S7).

We next derive the buffer equilibrium ratio using this definition of the buffer variable.

The forward and reverse reaction rates of

$$g_y = k_y z_m y, \quad g_x = k_x z_a^2,$$

and the equilibrium is

$$K = \frac{k_x}{k_y} = \frac{z_m y}{z_a^2}.$$

For $A = y + x_a$, we have

$$(A - x_a)\bar{y} = K(x_a - y)^2$$

resulting in

$$x_a = \left(1 - \frac{1}{2K}\right) y \pm \sqrt{\frac{A}{K} y + \left(\left(1 - \frac{1}{2K}\right)^2 - 1\right) y^2}$$

As $x_a - y \geq 0$, this simplifies to

$$x_a = \left(1 - \frac{1}{2K}\right) y + \sqrt{\frac{y^2}{4K^2} + \frac{y}{K}(A - y)} \tag{S43}$$

SI4.3.1 Fixed parameters K and A

Differentating (S43), we have

$$B_a^f = \frac{dx_a}{dy}\Big|_{y=\bar{y}} = \left(1 - \frac{1}{2K}\right) + \frac{\frac{\bar{y}}{2K^2} + \frac{A}{K} - \frac{2\bar{y}}{K}}{2\sqrt{\frac{\bar{y}^2}{4K^2} + \frac{\bar{y}}{K}(A - \bar{y})}}$$

Substituting from (S43), we have

$$B_a^f = \frac{(A - \bar{x}) + 2K_a(\bar{x} - \bar{y})}{\bar{y} + 2K_a(\bar{x} - \bar{y})} = \frac{[AMP] + 2K_a[ADP]}{[ATP] + 2K_a[ADP]}$$

where $[ATP]$, $[ADP]$, $[AMP]$ are evaluated at the set point. Thus we have

$$\boxed{B_a^f = \frac{[AMP] + 2K_a[ADP]}{[ATP] + 2K_a[ADP]}}$$

which is shown in (9) in the main section.

SI4.3.2 Varying parameters K and A

We next wish to determine the effect of varying K and A on buffer equilibrium ratio B. From (S43), we have

$$x_a = \left(1 - \frac{1}{2K_a}\right) y + \sqrt{\frac{y^2}{4K_a^2} + \frac{y}{K_a}(A - y)}$$

For varying A, we have

$$\begin{aligned}
\frac{\partial x_a}{\partial A}\Big|_{y=\bar{y}} &= \frac{1}{2}\left(\frac{\bar{y}^2}{4K_a^2} + \frac{\bar{y}}{K_a}(A - \bar{y})\right)^{-1/2} \frac{\bar{y}}{K_a} \\
&= \frac{\bar{y}}{\bar{y} + 2K_a(\bar{x} - \bar{y})} \\
&= \frac{\bar{y}}{(A - \bar{x}) + 2K_a(\bar{x} - \bar{y})} B_a^f \\
&= \frac{A}{(A - \bar{x}_a) + 2K_a(\bar{x}_a - \bar{y})} \frac{\bar{y}}{A} B_a^f
\end{aligned}$$

where

$$B_a^f = \frac{(A - \bar{x}_a) + 2K_a(\bar{x}_a - \bar{y})}{\bar{y} + 2K_a(\bar{x}_a - \bar{y})}.$$

This results in

$$B_a = B_a^f \left(1 + \frac{\bar{y}}{(A - \bar{x}_a) + 2K_a(\bar{x}_a - \bar{y})} \frac{dA}{dy}\right) \tag{S44}$$

$$\tag{S45}$$

where dA/dy is evaluated at $y = \bar{y}$. This can be rewritten

$$\boxed{B_a = B_a^f \left(1 + \frac{[ATP]}{[AMP] + 2K_a[ADP]} \frac{dA}{dy}\right)} \tag{S46}$$

which is shown in (10) in the main section.

The adenine nucleotide total, A, typically decreases with decreasing ATP ($R_m > 0$) and so the effect of AMPD changing A is to synergistically increase B_a in (7).

If we also have K that varies with y then we can also include extra terms in B_a. We have

$$\frac{\partial x_a}{\partial K_a}\Big|_{y=\bar{y}} = \frac{\bar{y}}{2K_a^2} - \frac{1}{2}\left(\frac{\bar{y}^2}{4K_a^2} + \frac{\bar{y}}{K_a}(A-\bar{y})\right)^{-1/2}\left(\frac{\bar{y}^2}{2K_a^3} + \frac{\bar{y}}{K_a^2}(A-\bar{y})\right).$$

Substituting, we have

$$\begin{aligned}\frac{\partial x_a}{\partial K_a} &= \frac{\bar{y}}{2K^2} - \frac{1}{2}\frac{\frac{\bar{y}^2}{2K_a^3} + \frac{\bar{y}}{K_a^2}(A-\bar{y})}{\bar{x}_a - \left(1 - \frac{1}{2K_a}\right)\bar{y}} \\ &= -\frac{A-\bar{x}_a}{A-\bar{x}_a+2K_a(\bar{x}_a-\bar{y})}\frac{\bar{y}}{K}B_{fixed}\end{aligned}$$

which can be rewritten

$$\frac{\partial x_a}{\partial K_a} = -\frac{([AMP]+2K_a[ADP])}{[AMP]}\frac{[ATP]}{K_a}B_a.$$

Combining with (S44), we have

$$B_a = B_a^f\left(1 - \frac{A-\bar{x}_a}{A-\bar{x}_a+2K_a(\bar{x}-\bar{y})}\frac{y}{K_a}\frac{dK_a}{dy} + \frac{\bar{y}}{(A-\bar{x}_a)+2K_a(\bar{x}_a-\bar{y})}\frac{dA}{dy}\right)$$

where dA/dy and dK/dy are evaluated at $y=\bar{y}$. This can be rewritten

$$B_a = B_a^f\left(1 - \frac{[AMP]}{[AMP]+2K_a[ADP]}\frac{ATP}{K_a}\frac{\Delta K_a}{\Delta ATP} + \frac{[ATP]}{[AMP]+2K_a[ADP]}\frac{\Delta A}{\Delta ATP}\right).$$

SI4.3.3 Comparison to Experimental Data

We next compare the theoretical and experimental values for B_a in human muscles and adypocyte during exercise. To determine the experimental B_a, observations of ATP and ADP over time (e.g. before and after a period of exercise) can be used in the formula

$$B_a = \frac{\Delta([ATP]+[ADP])}{\Delta[ATP]} = \frac{[ATP]_{final}+[ADP]_{final}-[ATP]_{initial}-[ADP]_{initial}}{[ATP]_{final}-[ATP]_{initial}}. \tag{S47}$$

To calculate the theoretical B over a range of y, we use

$$B_a = \frac{\Delta x}{\Delta y} = \frac{1}{\Delta y}\int_{y_0}^{y_1} B(y)dy \tag{S48}$$

where $\Delta y = y_1 - y_0$. We know experimental values of K_a, y/D for particular measurement points rather than all values, and so for fixed parameter B_a^f we approximate the integral using the trapezoidal rule

$$B_a^f = \frac{1}{\Delta y}\sum_k \frac{1}{2}[B(y_{k+1})+B(y_k)](y_{k+1}-y_k). \tag{S49}$$

The theoretical B is estimated from the experimental K_a, y/D at each data point.

As above, we use the geometric average as parameters can vary over orders of magnitude. If we set

$$B_a = B_a^f + B_a^K + B_a^A, \quad B_a^K = -B_a^f\left(1-\frac{y}{D}\right)\frac{y}{K_a}\frac{dK_a}{dy}, \quad B_a^A = -B_a^f\frac{y}{D}\frac{dD}{dy} \tag{S50}$$

then

$$
\begin{aligned}
B_a =& \frac{1}{\Delta y}\sum_k \frac{1}{2}[B_a^f(y_{k+1}) + B_a^f(y_k)](y_{k+1} - y_k) \\
&+ \frac{1}{\Delta y}\sum_k S_K\sqrt{|B_K(y_{k+1})B_K(y_k)|}(y_{k+1} - y_k) \\
&+ \frac{1}{\Delta y}\sum_k S_A\sqrt{|B_A(y_{k+1}) + B_A(y_k)|}(y_{k+1} - y_k)
\end{aligned}
\tag{S51}
$$

where $S_K = \text{sign}(B_K(y_{k+1})B_K(y_k))$ and $S_A = \text{sign}(B_A(y_{k+1})B_A(y_k))$.

B_{exp}	B_{theor}	B_{theor} inc. $\frac{dA}{dy}$	Ref	Tissue
0.94±0.09	0.11	0.96	[30](ATP ↓)	muscle
0.94±0.05	0.05	0.94	[19](ATP ↓)	muscle
0.85±0.09	0.05	0.84	[19](ATP ↑)	muscle
0.85±0.27	0.13	0.87	[10](ATP ↓)	muscle
0.88±0.41	0.09	0.88	[10](ATP ↓)	muscle
0.89±0.07	0.08	0.90	[22](ATP ↓)	muscle
0.74±0.17	0.05	0.76	[22](ATP ↑)	muscle
0.88±0.10	0.44	0.89*	[21](ATP ↓)	adipose - basal
0.78±0.42	0.44	0.79*	[21](ATP ↑)	adipose - basal

Table S2: Buffer equilibrium ratio B_a of adenylate kinase for mouse and for human muscle during and after exercise. *also incorporates $\frac{dK}{dy}$.

SI5 Sensitivity Functions and Energy Ratio Regulation

In this section, we determine the sensitivity of the ATP/ADP ratio, the ATP, AMP ratio and the energy charge to changes in ATP. We also compare these sensitivities to experimental data.

SI5.1 Sensitivity function: ATP/ADP Ratio

We next determine the sensitivity of the ATP/ADP Ratio to changes in ATP. We have the output

$$
y_d = \frac{[ATP]}{[ADP]} = \frac{y}{x_a - y}. \tag{S52}
$$

Differentiating, we have

$$
\Delta y_d = \frac{\bar{x}_a}{(\bar{x}_a - \bar{y})^2}\Delta y - \frac{\bar{y}}{(\bar{x}_a - \bar{y})^2}\Delta x_a.
$$

where the derivatives are evaluated at the steady state $(y, x) = (\bar{y}, \bar{x})$. Using $\Delta x_a = B_a \Delta y$, we have

$$
\Delta y_d = \frac{\bar{x}_a - B_a\bar{y}}{(\bar{x}_a - \bar{y})^2}\Delta y.
$$

Normalising, we have

$$
\frac{\Delta y_d}{\bar{y}_d} = \left(1 + (1 - B_a)\frac{\bar{y}}{\bar{x}_a - \bar{y}}\right)\frac{\Delta y}{\bar{y}}.
$$

Rewriting, we have

$$\boxed{\Delta_{\%}\left[\frac{ATP}{ADP}\right]\Big/\Delta_{\%}ATP = 1 + (1 - \underbrace{B_a}_{\text{AK}})\frac{ATP}{ADP}} \tag{S53}$$

which is shown in (11) in the main section.

SI5.2 Sensitivity function: ATP/AMP Ratio

We next determine the sensitivity of the ATP/AMP Ratio to changes in ATP. We have the output

$$y_{rm} = \frac{[ATP]}{[AMP]} = \frac{y}{A - x_a}. \tag{S54}$$

Differentiating, we have

$$\Delta y_{rm} = \frac{1}{A - \bar{x}_a}\Delta y + \frac{\bar{y}}{(A - \bar{x}_a)^2}\Delta x_a - \frac{\bar{y}}{(A - \bar{x}_a)^2}\frac{dA}{dy}\Delta y.$$

where the derivatives are evaluated at the steady state $(y, x) = (\bar{y}, \bar{x})$. Using $\Delta x_a = B_a \Delta y$, we have

$$\Delta y_{rm} = \frac{A - \bar{x}_a + B_a\bar{y} - \bar{y}\frac{dA}{dy}}{(A - \bar{x}_a)^2}\Delta y$$

Normalising, we have

$$\frac{\Delta y_{rm}}{\bar{y}_{rm}} = \left(1 + B_a\bar{y}_{rm} - \bar{y}_{rm}\frac{dA}{dy}\right)\frac{\Delta y}{\bar{y}}.$$

Setting $R_m = \frac{dA}{dy}$ and rewriting, we have

$$\boxed{\Delta_{\%}\left[\frac{ATP}{AMP}\right]\Big/\Delta_{\%}ATP = 1 + (\underbrace{B_a}_{\text{AK}} - \underbrace{R_m}_{\text{AMPD}})\frac{ATP}{AMP}} \tag{S55}$$

which is shown in (11) in the main section.

SI5.3 Sensitivity function: Energy Charge

We next determine the sensitivity of the the Energy Charge Ratio to changes in $[ATP]$. The energy charge can be written

$$E_c = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]} = \frac{y + x_a}{2A}. \tag{S56}$$

Differentiating, we have

$$\Delta E_c = \frac{1}{2A}\Delta y + \frac{1}{2A}\Delta x - \frac{\bar{y} + \bar{x}}{2A^2}\frac{dA}{dy}\Delta y.$$

where the derivatives are evaluated at the steady state $(y, x) = (\bar{y}, \bar{x})$. Using $\Delta x = B\Delta y$, we have

$$\Delta E_c = \frac{1}{2A}\left(1 + B_a - \frac{y + x}{A}\frac{dA}{dy}\right)\Delta y.$$

Normalising, we have

$$\frac{\Delta E_c}{E_c} = (1 + B_a - 2E_c R_m)\,\frac{\bar{y}}{2E_c A}\,\frac{\Delta y}{\bar{y}}$$

where $R_m = \frac{dA}{dy}$. Rewriting, we have

$$\boxed{\Delta_{\%} E_c \Big/ \Delta_{\%} ATP = \left(1 + \underbrace{B_a}_{\text{AK}} - 2\,\underbrace{E_C R_m}_{\text{AMPD}}\right)\frac{1}{2E_c}\,\frac{ATP}{A}} \tag{S57}$$

which is shown in (11) in the main section.

SI5.4 Comparison to Experimental Data

We can compare the theory to experimental results, which can be seen in the main section. For an initial and final value of ATP, ADP, AMP, we have

$$\begin{aligned}
\Delta_{\%}\left[\frac{ATP}{ADP}\right] &= \Delta\left[\frac{ATP}{ADP}\right] \Big/ \frac{ATP}{ADP} \\
\Delta_{\%}\left[\frac{ATP}{AMP}\right] &= \Delta\left[\frac{ATP}{AMP}\right] \Big/ \frac{ATP}{AMP} \\
\Delta_{\%} E_c &= \Delta E_c \Big/ E_c
\end{aligned} \tag{S58}$$

where

$$\begin{aligned}
\Delta\left[\frac{ATP}{ADP}\right] &= \left[\frac{ATP}{ADP}\right]_{initial} - \left[\frac{ATP}{ADP}\right]_{final} \\
\Delta\left[\frac{ATP}{AMP}\right] &= \left[\frac{ATP}{AMP}\right]_{initial} - \left[\frac{ATP}{AMP}\right]_{final} \\
\Delta E_c &= [E_c]_{initial} - [E_c]_{final}
\end{aligned} \tag{S59}$$

and for the normalisation we use the geometric averages

$$\begin{aligned}
\frac{ATP}{ADP} &= \left(\left[\frac{ATP}{ADP}\right]_{initial}\left[\frac{ATP}{ADP}\right]_{final}\right)^{1/2} \\
\frac{ATP}{AMP} &= \left(\left[\frac{ATP}{AMP}\right]_{initial}\left[\frac{ATP}{AMP}\right]_{final}\right)^{1/2} \\
E_c &= ([E_c]_{initial}[E_c]_{final})^{1/2}
\end{aligned} \tag{S60}$$

To determine the theoretical results, we substitute the geometric averages of the experimental values for B_a, R_m, ATP/ADP, ATP/AMP, and E_c into Equations (S53), (S55) and (S57).