Systembiologie 551-1174-00L

Metabolic Regulation

16 March, 2017

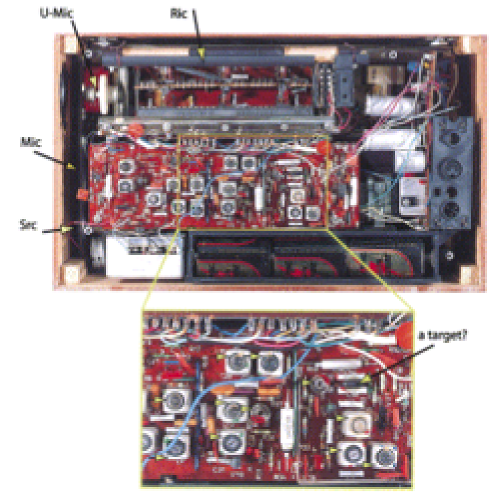
Uwe Sauer & Jörg Stelling

Content:

- Metabolic regulation mechanisms & mechanistic interpretation of metabolite data
- Can engineers understand glycolytic control?
- Can a biologist improve control of glycolysis?

Can Engineers Understand Metabolic Control?

"Conceptually, a radio functions similar to a signal transduction pathway; i.e. it converts a signal from one form into another. It has about 100 components - so somewhat similar to some biological systems."



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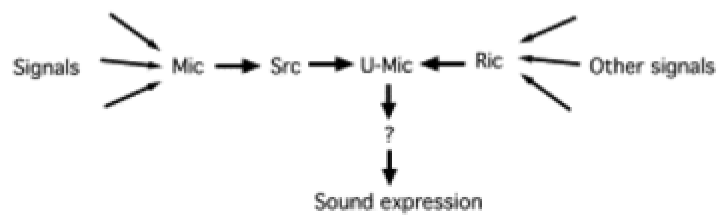

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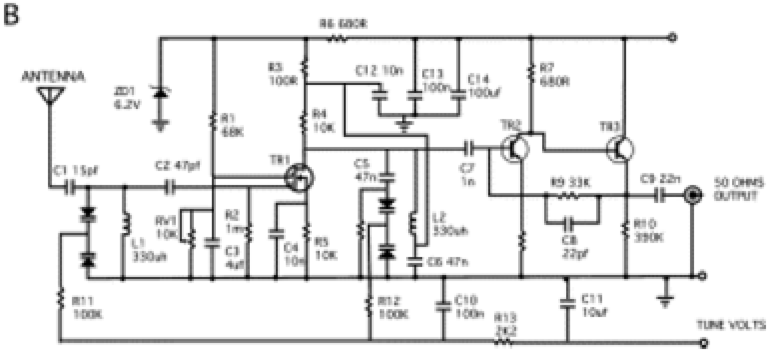
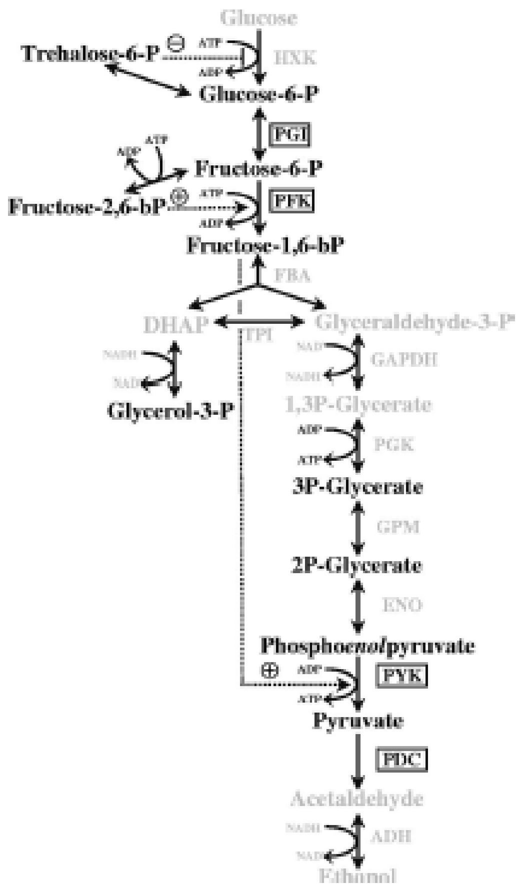


- **'Inverse' question:** Can standardized, quantitative language and models help understand biological systems?

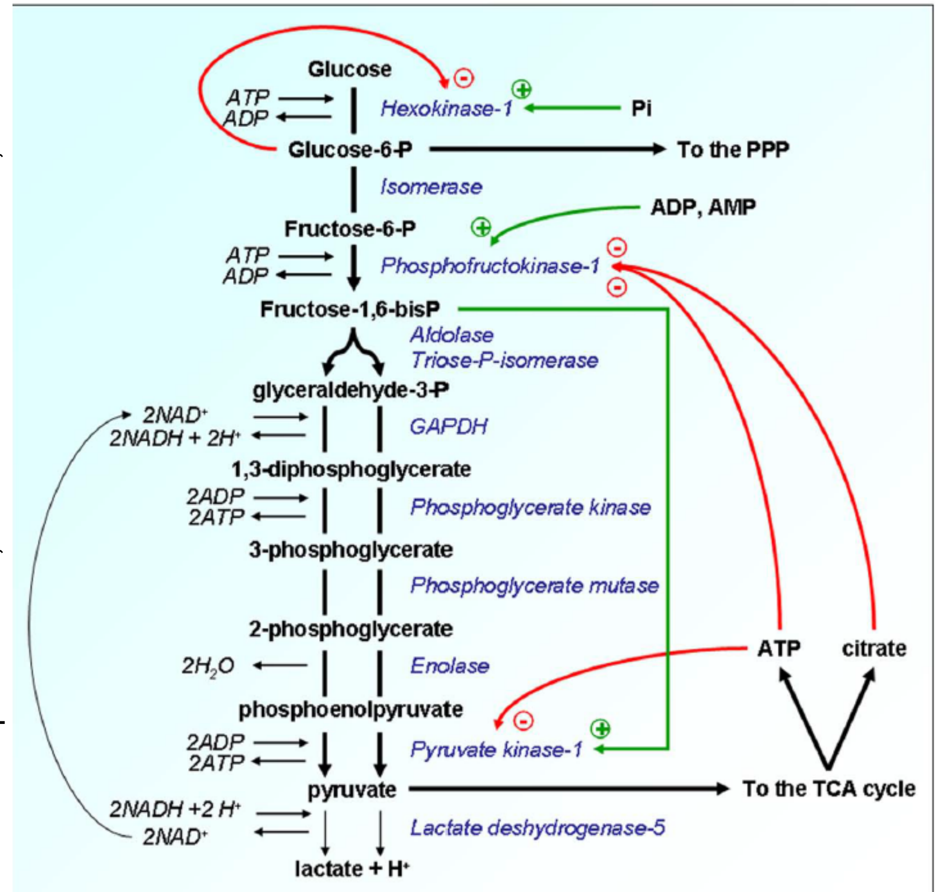
Y. Lazebnik, 2002. Can a biologist fix a radio? *Cancer Cell Biology* 2: 179

Glycolysis in Eukaryotes is too Complicated

J. van den Brink et al., *Appl. Env. Microbiol.* 74: 5710, 2008



P. Porporato et al., *Frontiers Pharmacol.* 2: 49, 2011



- Glycolytic regulation: There is not a single (biological) model → Standardize and focus.

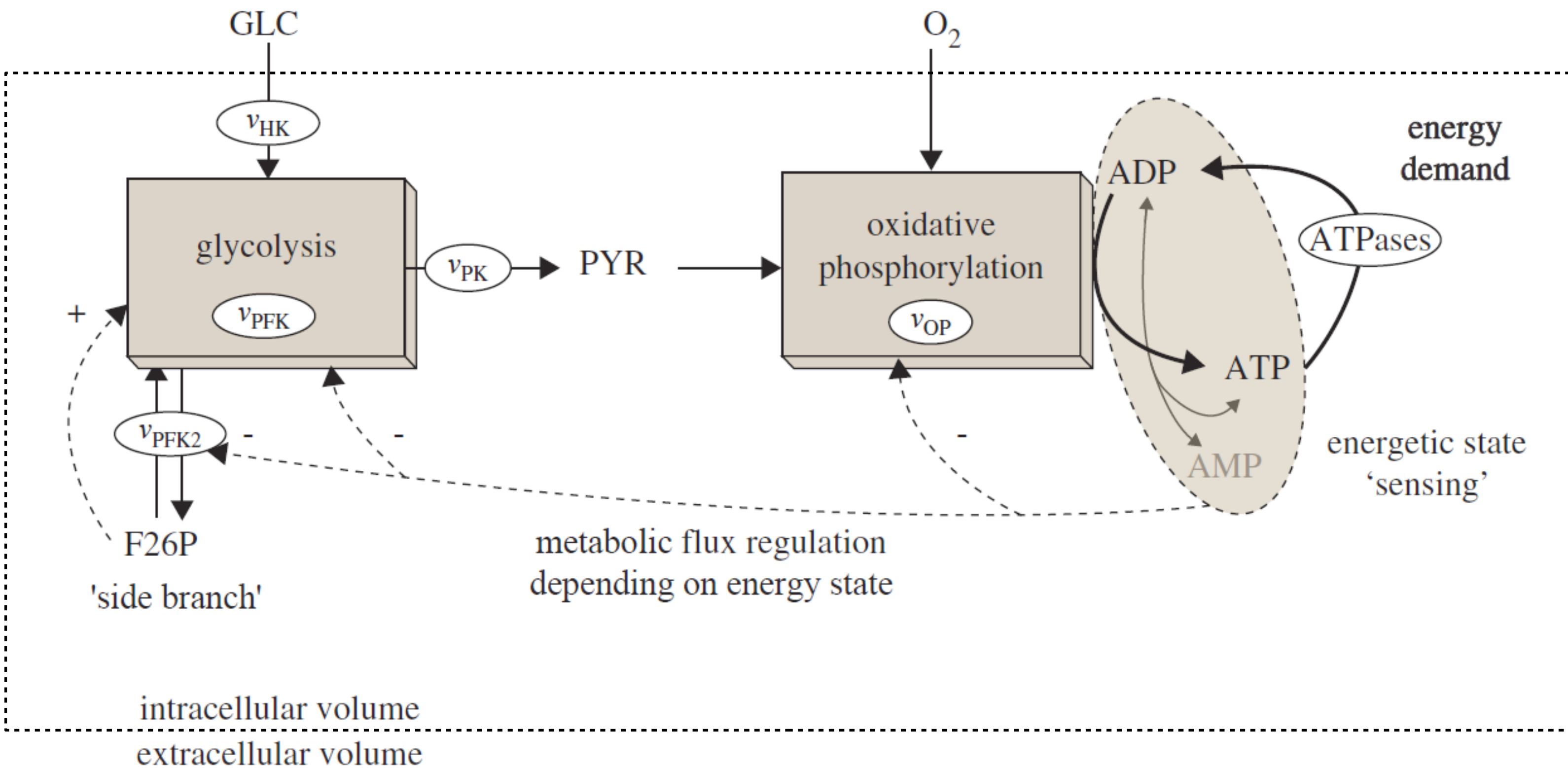
Aims of Regulation of Energy Metabolism?

- Stable dynamic behavior: Steady-state (no oscillations or chaotic behaviors).

- Fixed ATP concentration in the cell (despite fluctuating nutrient supply and energy demand).

- Tight dynamic control: Fast responses to perturbations (but without over- or undershoots).

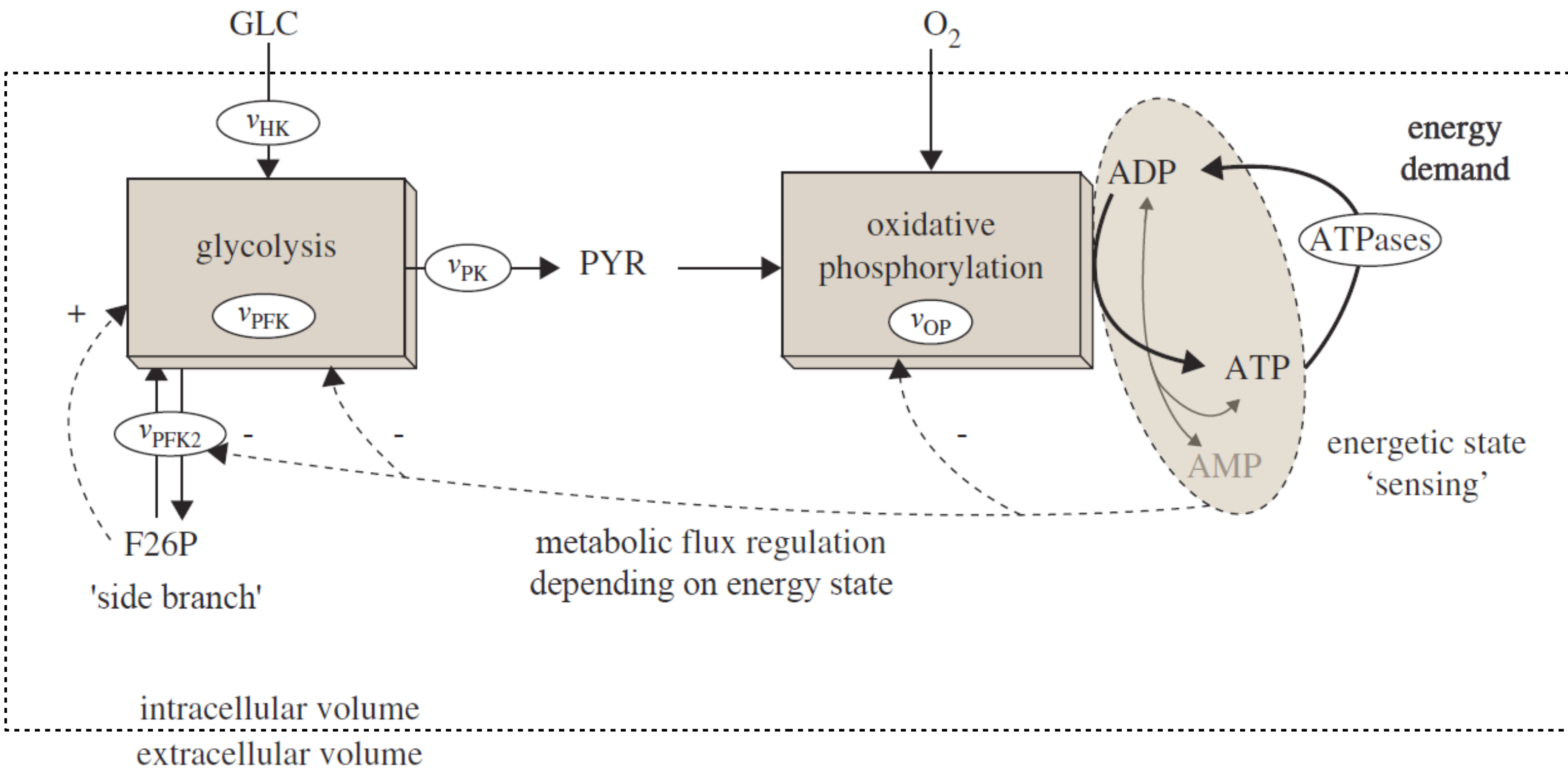
A Simplified Model of Energy Metabolism



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- How does the eukaryotic cell achieve energy (ATP) homeostasis despite fluctuating demand?

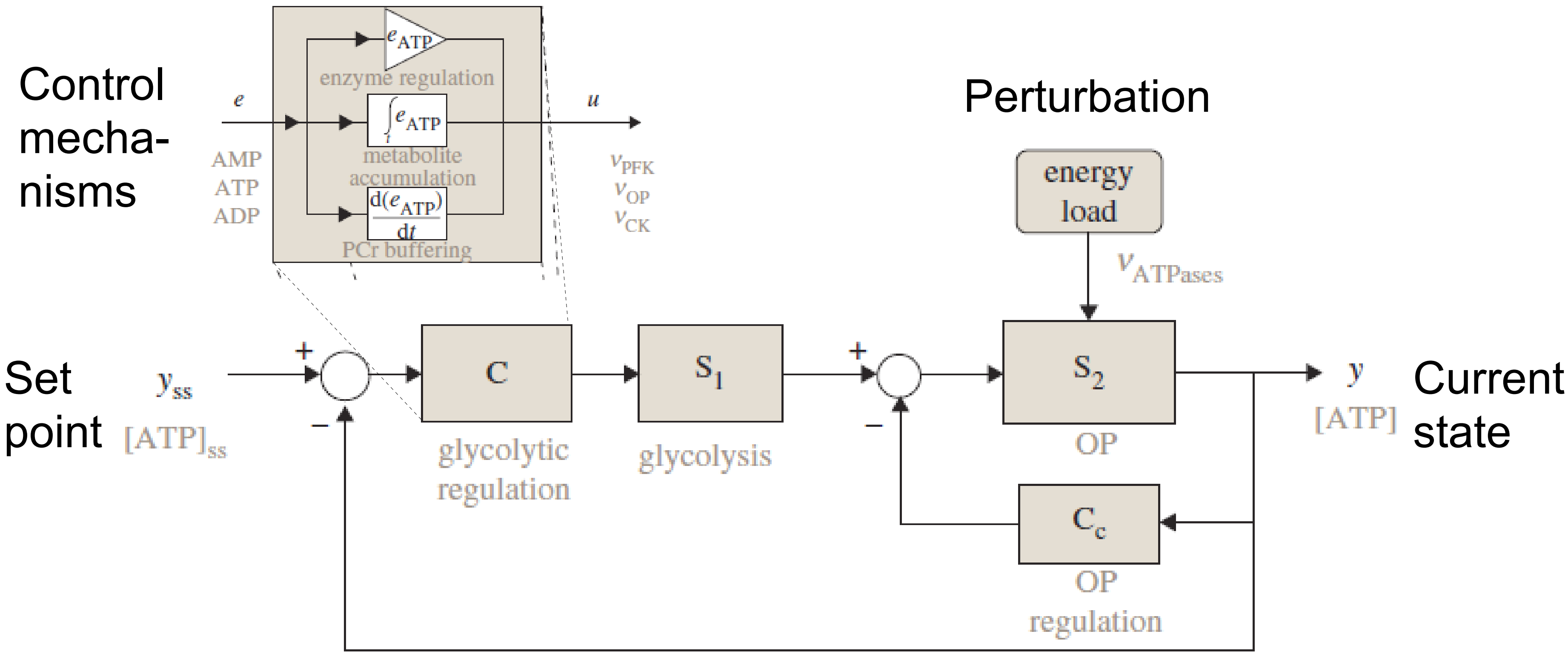
A Simplified Model of Energy Metabolism



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- **Answer:** Feedback. But why so many feedback loops (evolution, robustness, function, ...)?

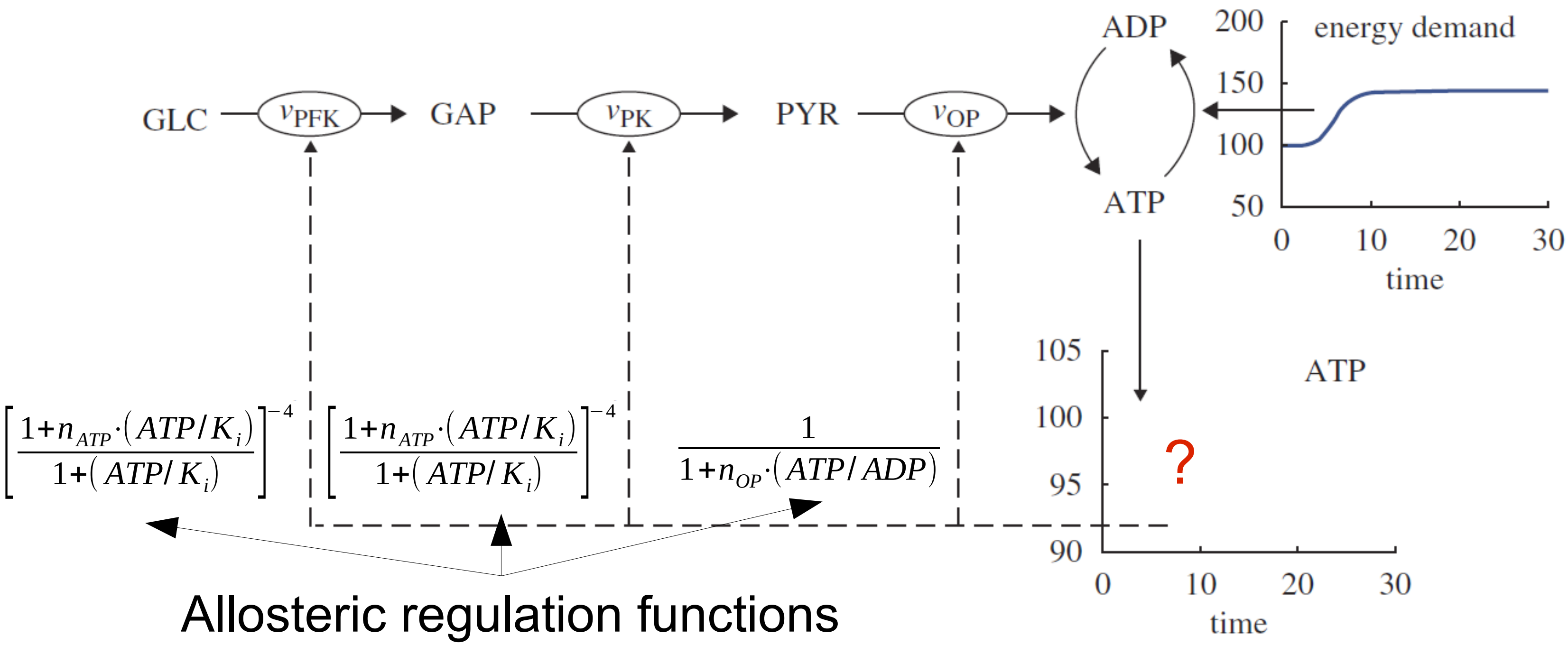
An Engineer's View: Block Diagram



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- **Answer:** Feedback. But different types of (negative) feedback have different functions.

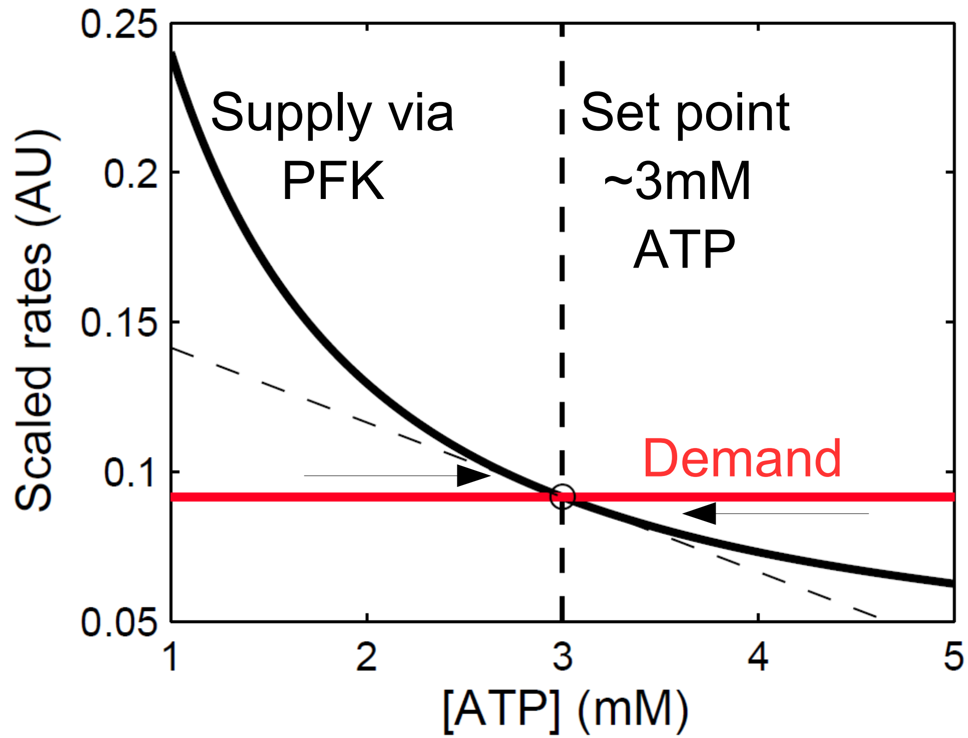
What Does Allosteric Regulation Achieve?



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

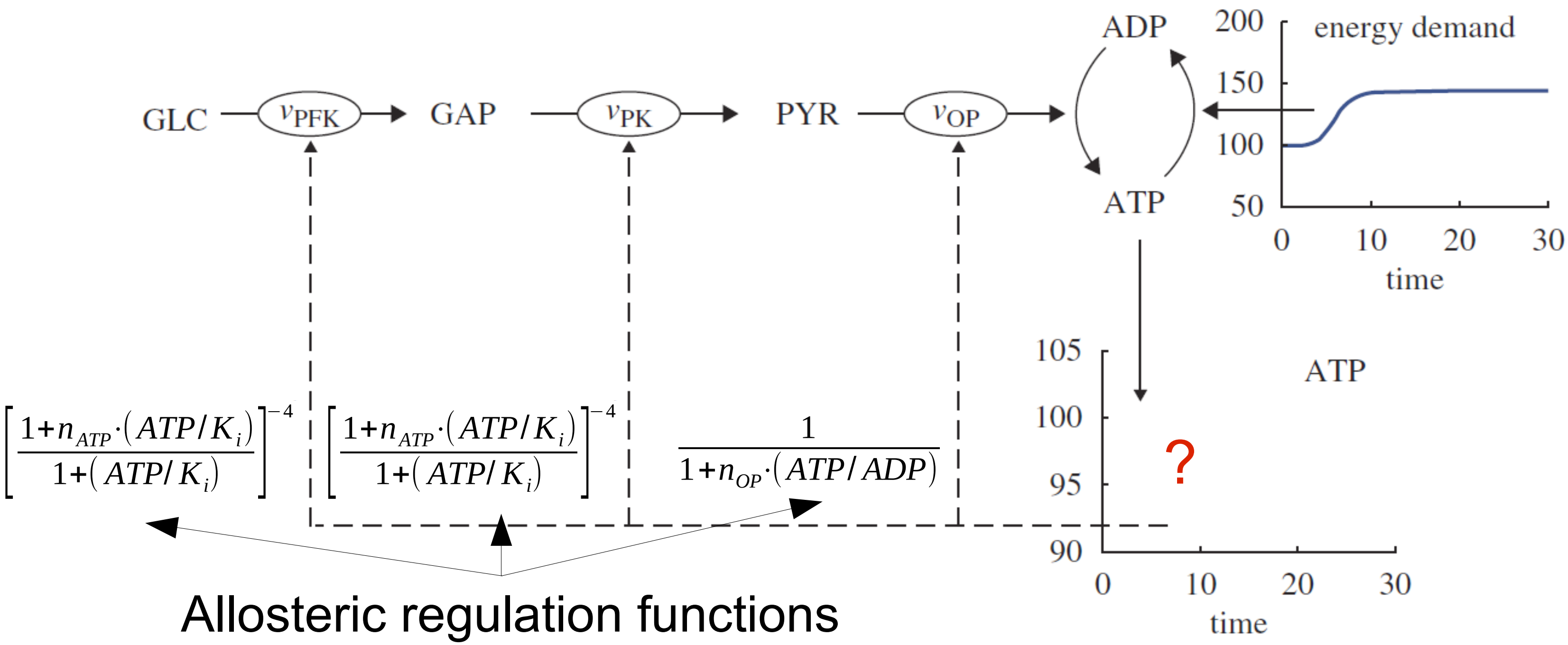
- Allosteric regulation establishes negative feedback on pathway flux depending on the current ATP concentration.

What Does Allosteric Regulation Achieve?



- Allosteric inhibition of flux via PFK: $v_{PFK} \propto \left[\dfrac{1+n_{ATP}\cdot(ATP/K_i)}{1+(ATP/K_i)}\right]^{-4}$.
- Approximately **proportional** negative feedback on ATP.

What Does Allosteric Regulation Achieve?

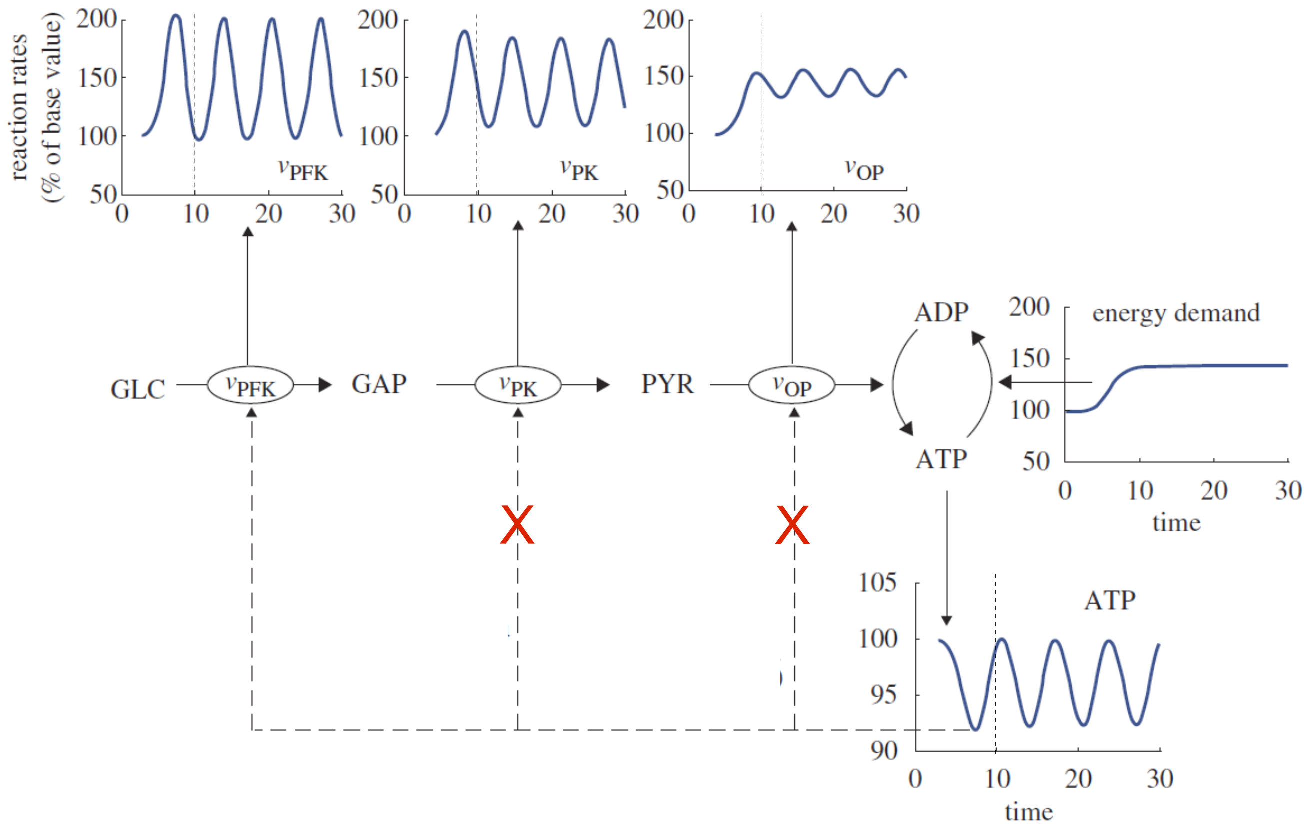


Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- ❑ Why three different control targets in energy metabolism?
- ❑ How to test the contributions of individual feedback loops?

What Does Allosteric Regulation Achieve?

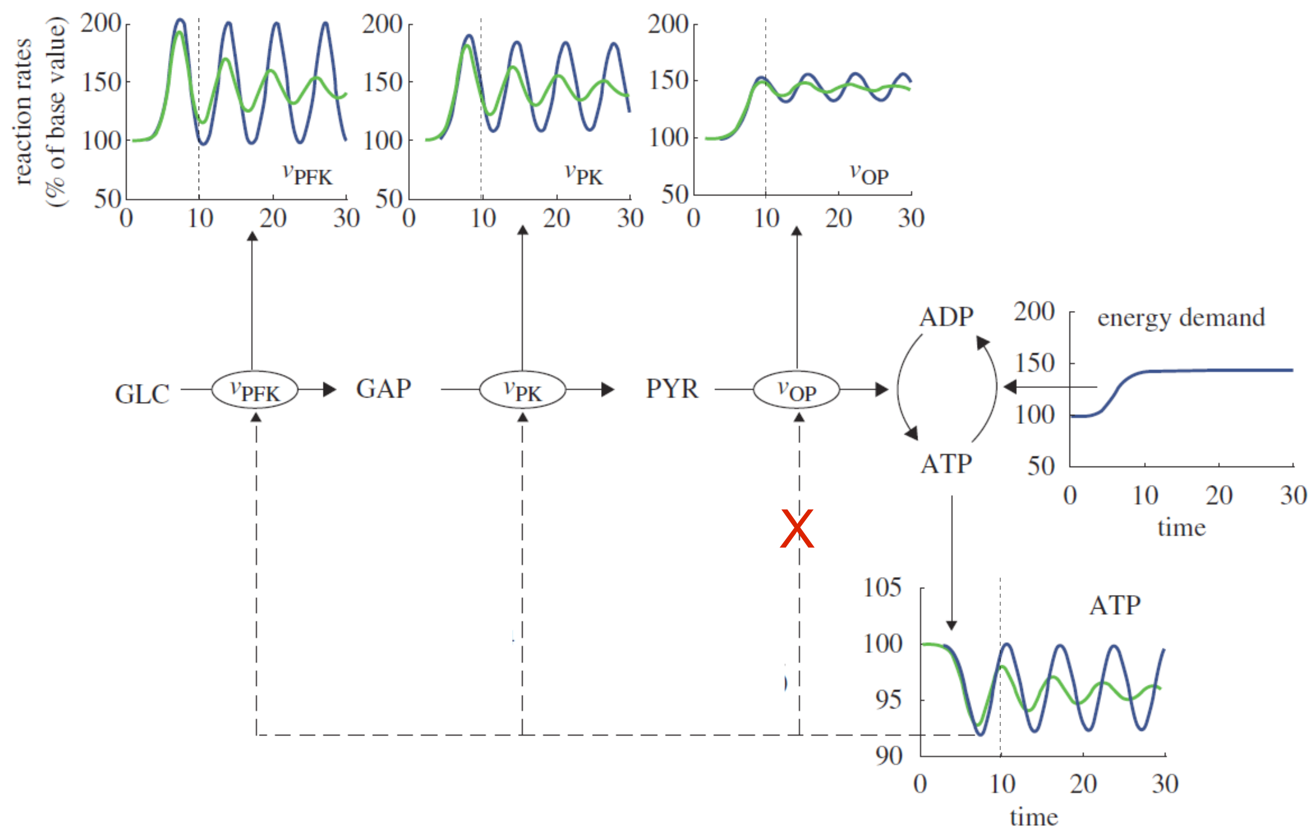
Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.



- Test of individual feedbacks by *in silico* knock-outs.
- Only 'long' PFK feedback → Time delay → Instabilities!

What Does Allosteric Regulation Achieve?

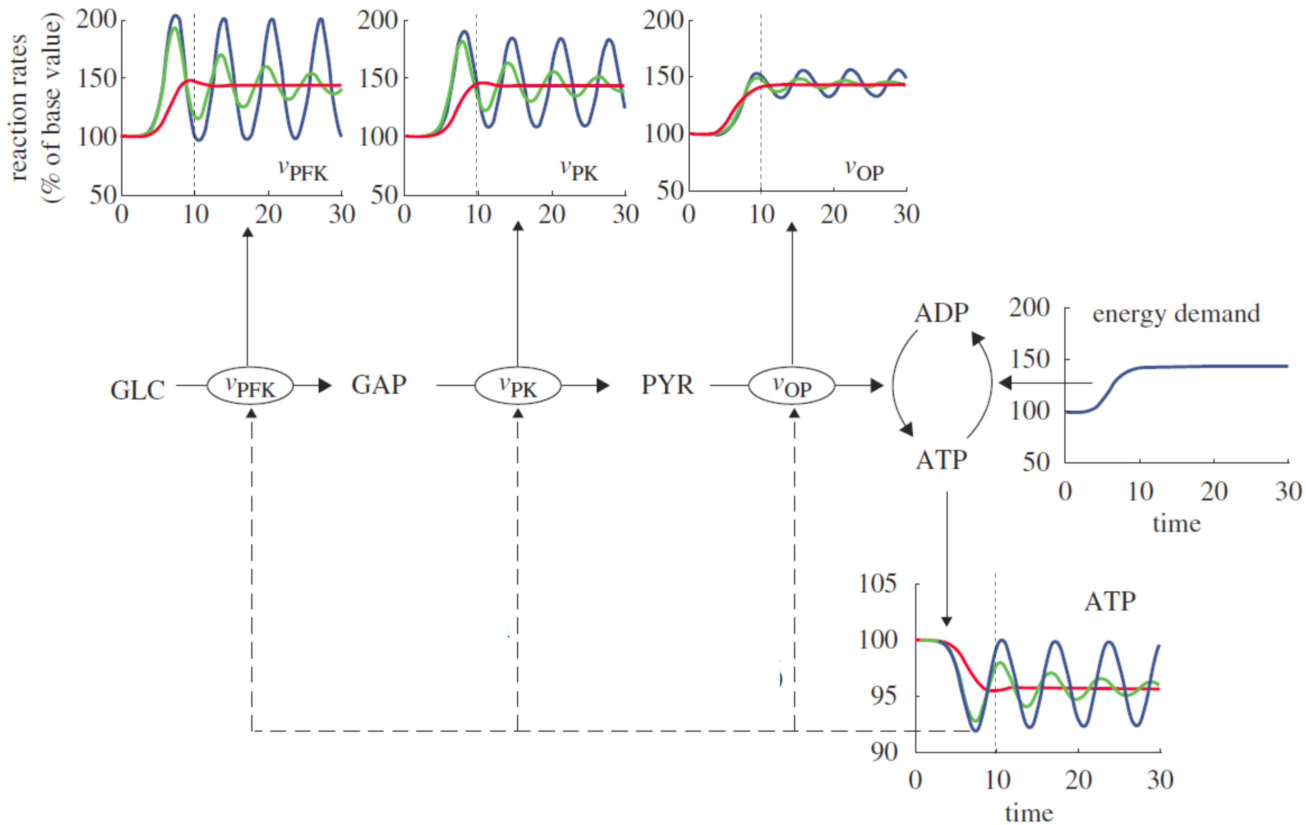
Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.



- Test of individual feedbacks by *in silico* knock-outs.
- Two (cascaded) feedbacks → Reduced instabilities.

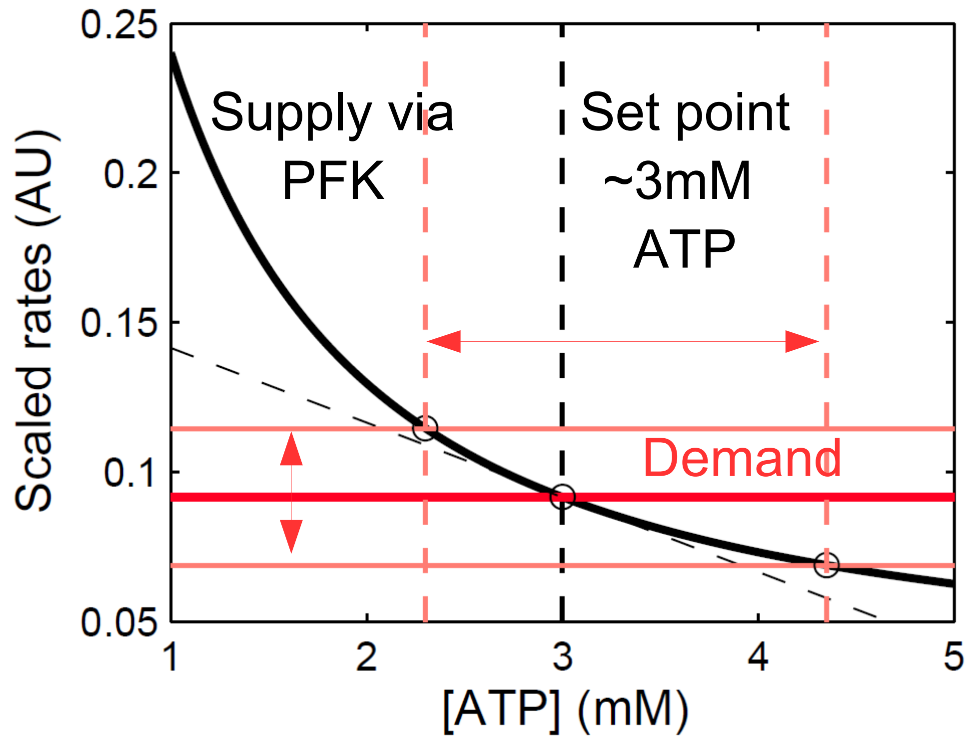
What Does Allosteric Regulation Achieve?

Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.



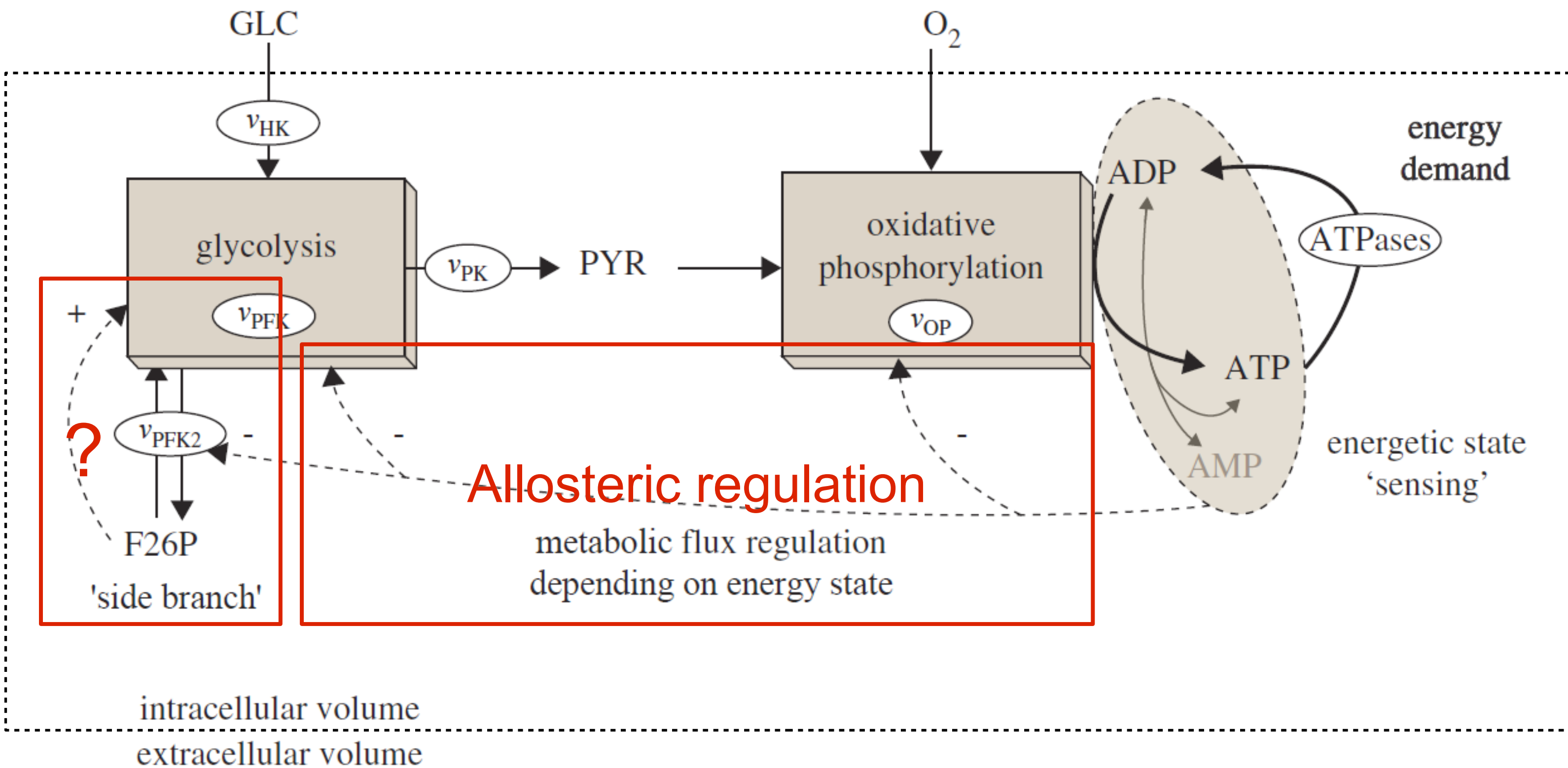
- Three (cascaded) feedbacks → Homeostasis achieved.
- But what is biologically 'wrong' with the ATP response?

Limitations of Proportional Feedback



- **Problem:** Proportional feedback does not lead to the **same** steady-state ATP concentration for **different** demands → Large deviations from set point possible.

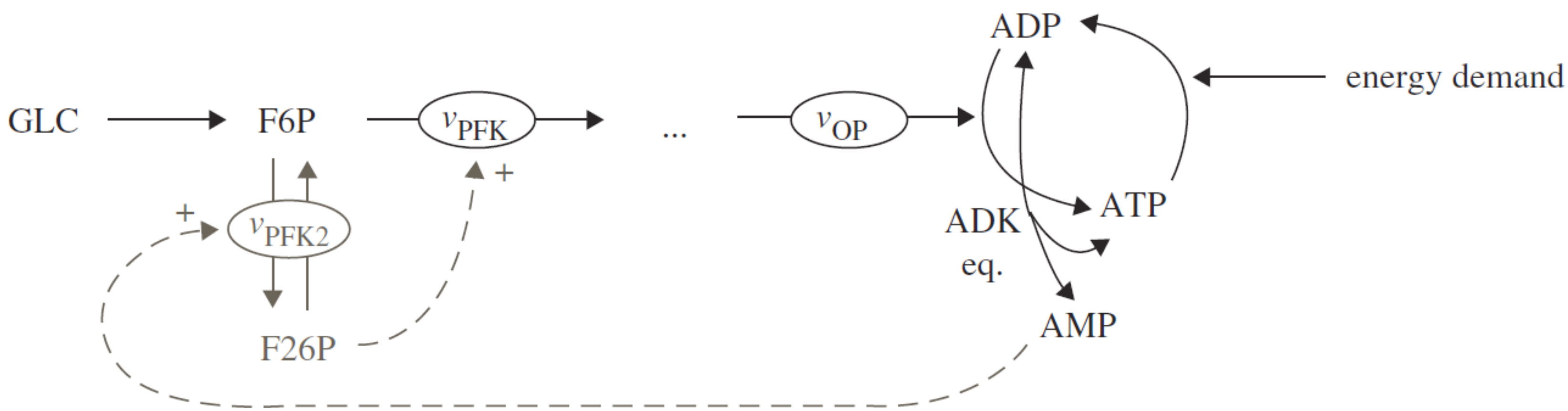
Back to the Simplified Model



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

'Glycolysis is usually presented as a linear pathway with nine reactions. However, this representation neglects a very important side reaction, the PFK2. This reaction allows the accumulation of F26P, one of the strongest activators of glycolysis.'

Feedback Regulation by PFK2

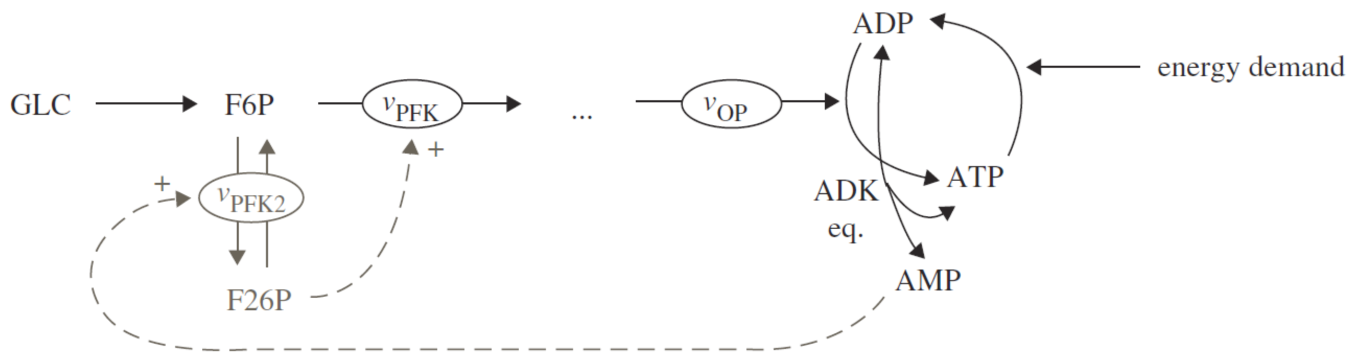


Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- AMP activates F26P production, which activates PFK → Another feedback loop in the system.

- Which type of feedback? Why different from the allosteric feedback loops already discussed?

Integral Negative Feedback by PFK2



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

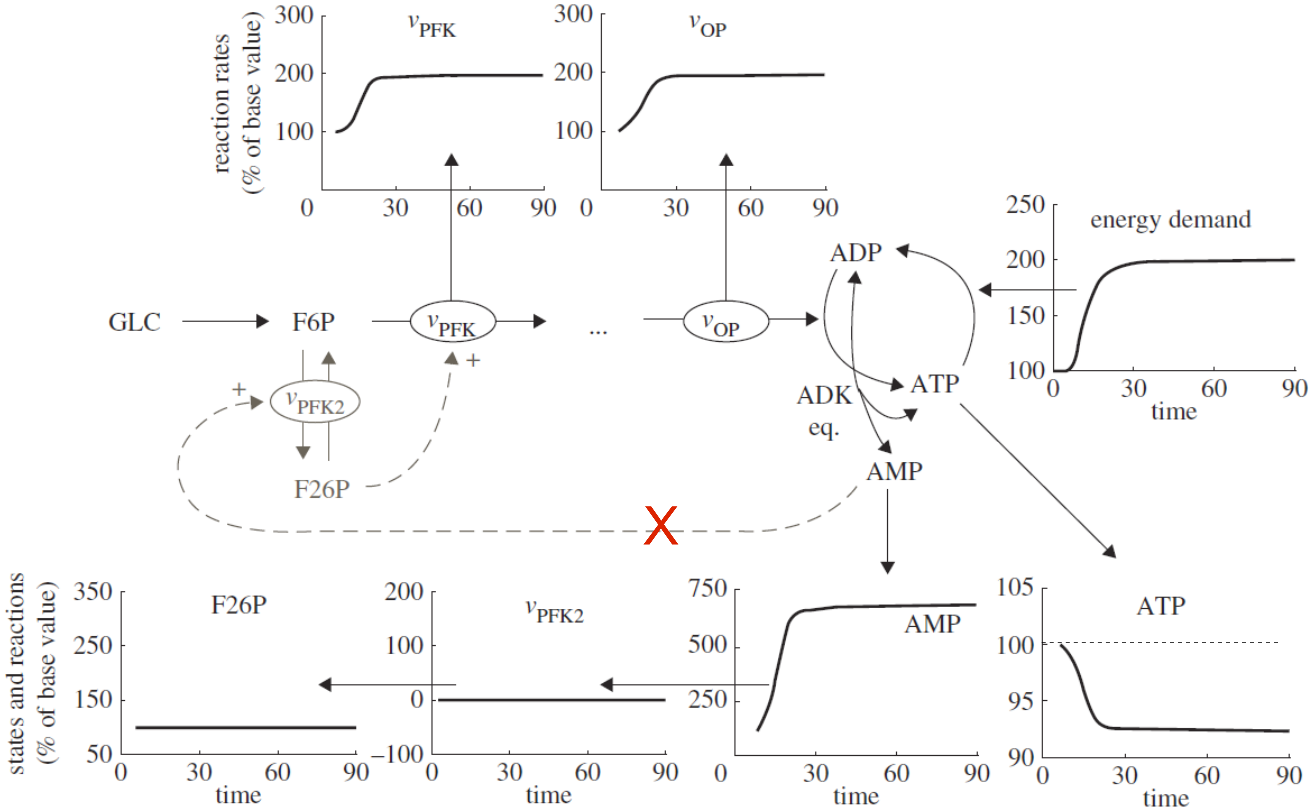
- If total AXP is conserved then $[AMP]$ represents the '$error$' in the current energy (ATP) state.

- The negative feedback loop **integrates** the error:

$$\frac{d[F26P]}{dt} = v_{PFK2} \propto error \Rightarrow v_{PFK} \propto [F26P] \propto \int_t error$$

Integral Negative Feedback by PFK2

Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.



- Without integral feedback: Deviation from ATP set-point.

Integral Negative Feedback by PFK2

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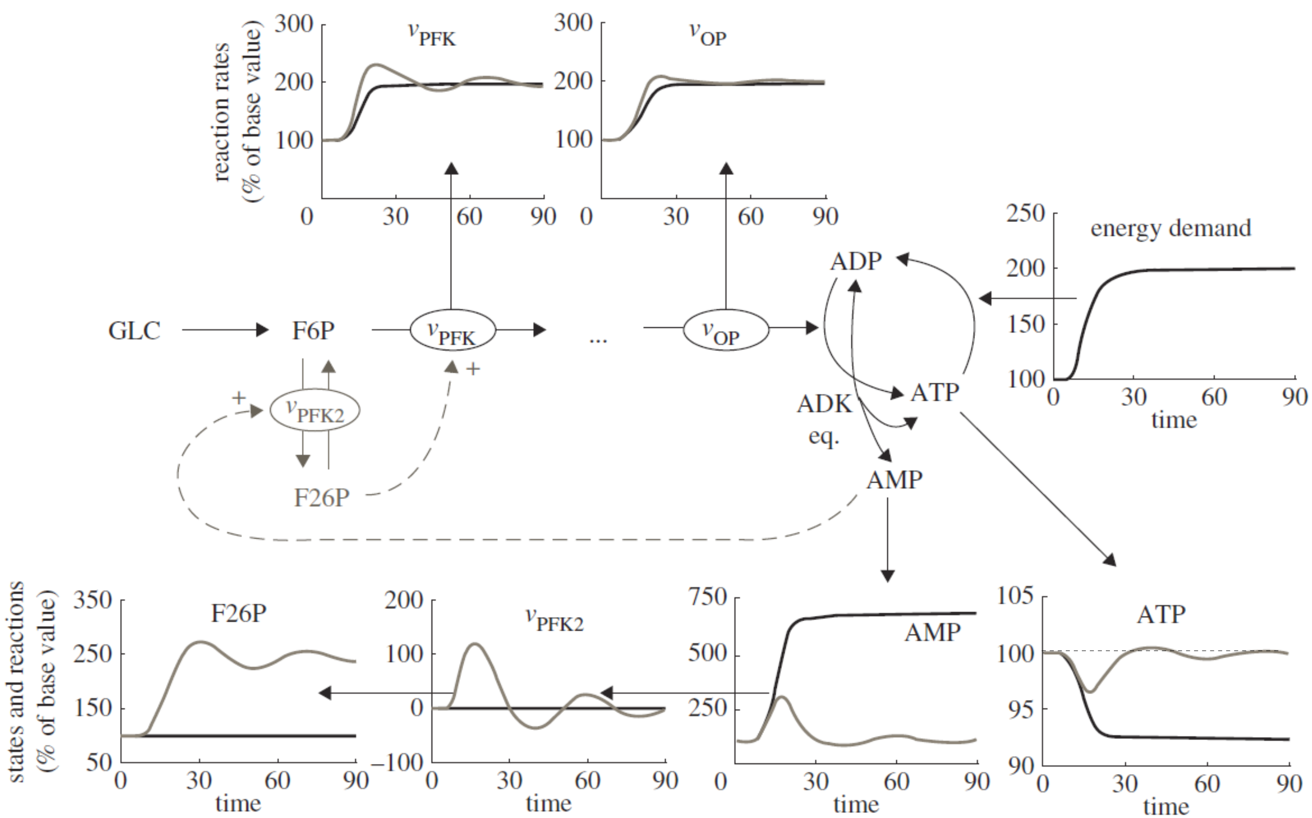
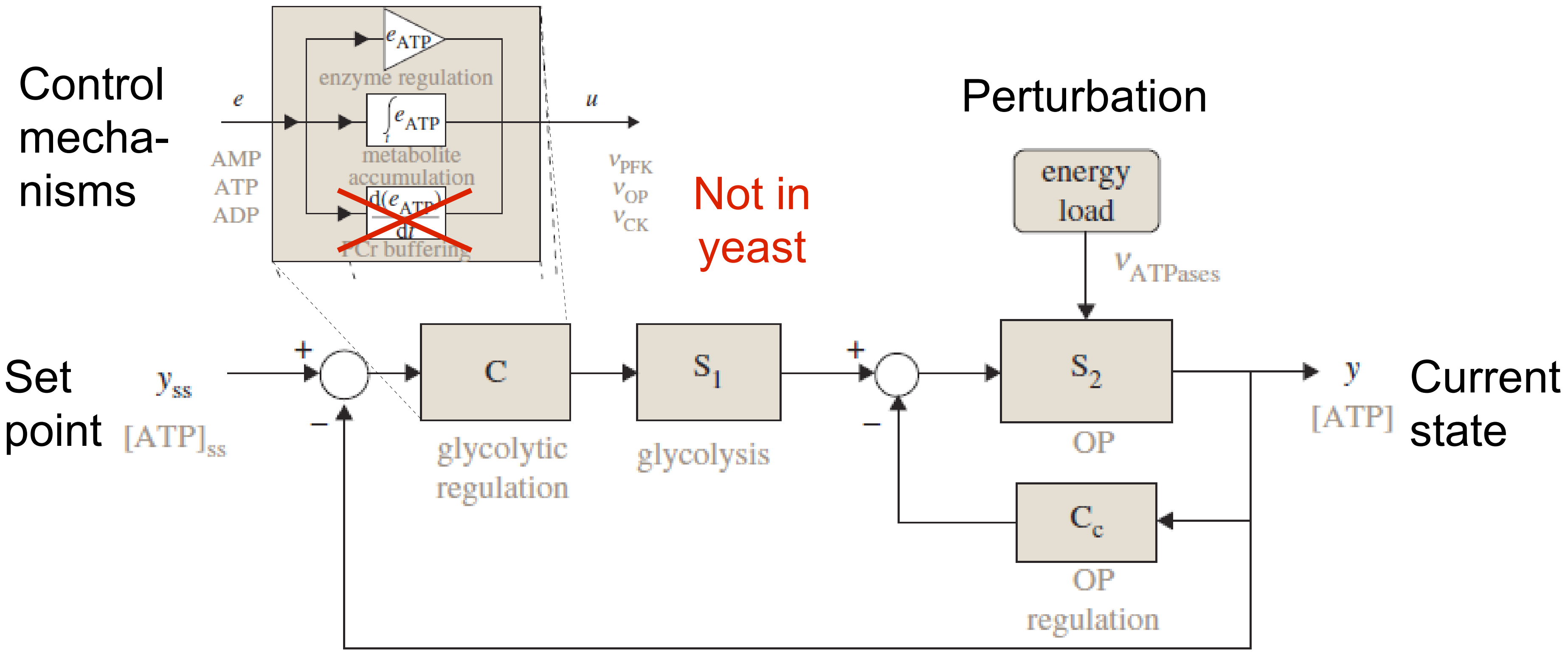


- Without integral feedback: Deviation from ATP set-point.
- With integral feedback: **Adaptation** to varying demand.

Summary: An Engineer's View



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- Control principles help understanding biological regulation.
- Unfortunately yeast is not perfect → Improvements?

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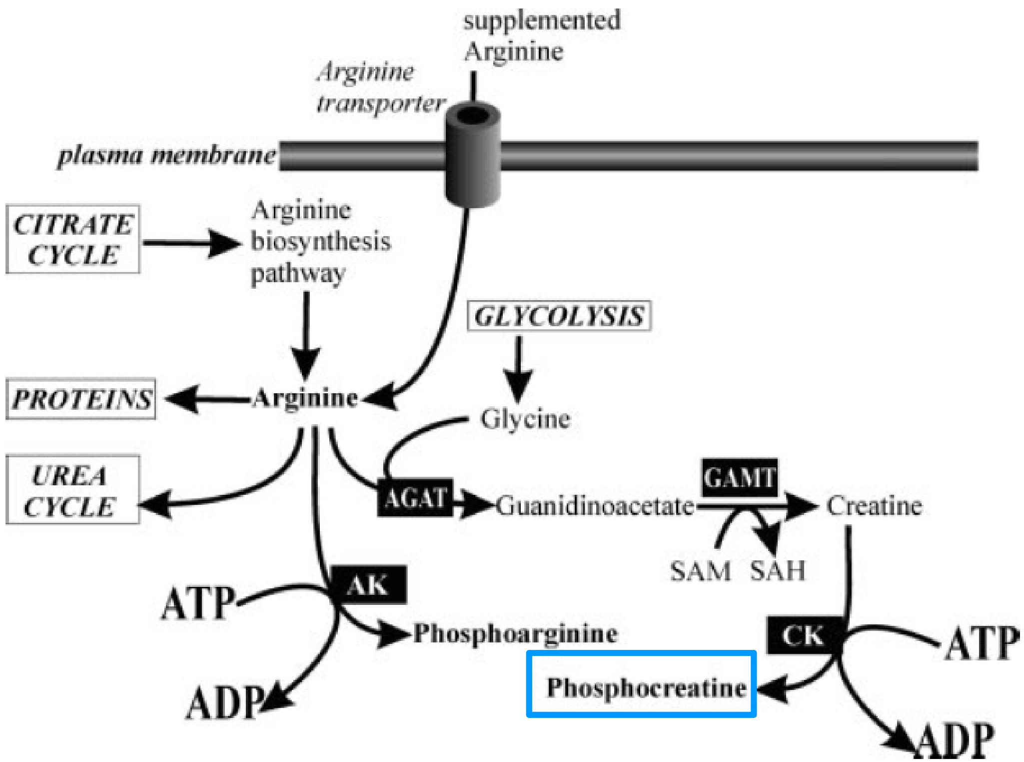
Engineering Phosphocreatine into Yeast

Figure from F. Canonaco et al., *J. Biol. Chem.* 277: 31303, 2002.

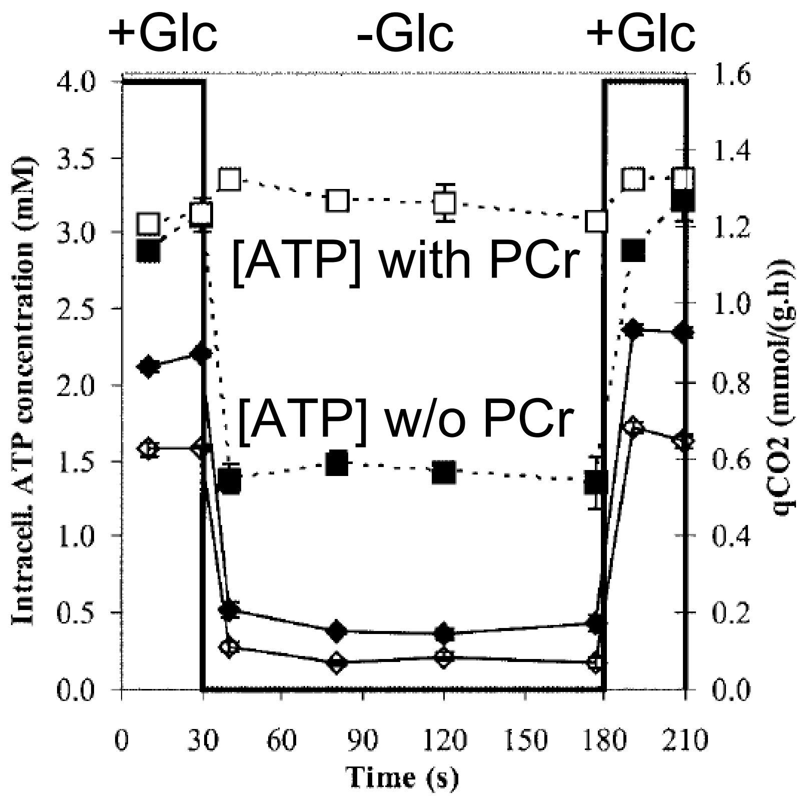


- Phosphocreatine (PCr): Metabolically inert equivalent of ATP → Buffer for replenishing ATP in mammals.

$$MgADP^{-} + PCr^{2-} + H^{+} \rightleftarrows MgATP^{2-} + Cr$$

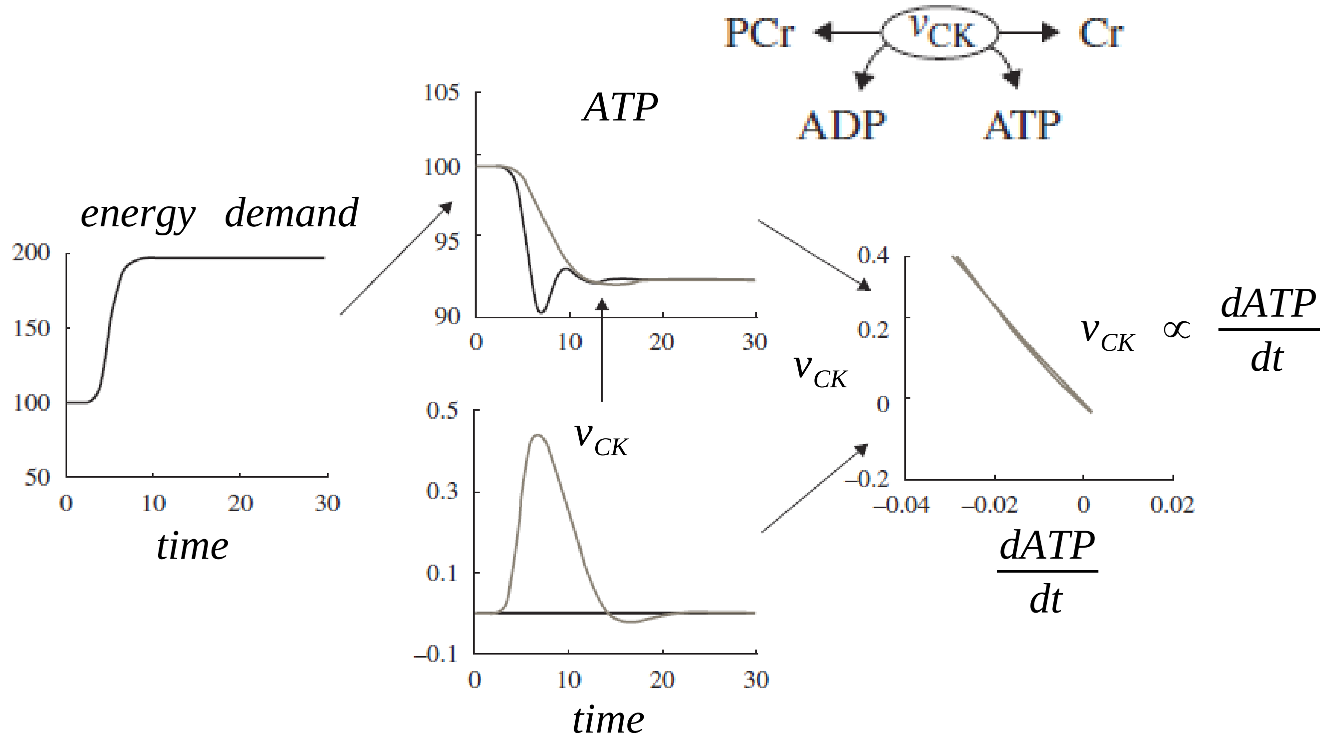
Engineering Phosphocreatine into Yeast

Figure from F. Canonaco et al., *J. Biol. Chem.* 277: 31303, 2002.



- ❑ Phosphocreatine (Pcr) engineered yeast maintains the physiological ATP level during sudden starvation.

Metabolic Buffers for Differential Control



Adapted from M. Cloutier & P. Wellstead, *J. R. Soc. Interface*, 2009.

- Buffers respond to the rate of change (**differential**) of the perturbation → Compensation for rapid changes.

Teaching Goal III: Summary

- Cellular regulation involves many feedbacks because the cell has many control objectives. Which objectives?
- Multiple feedback loops can help prevent undesired system dynamics such as oscillations. How?
- Different types of negative feedback can be implemented in biological circuits to achieve specific behaviors / functions.
  Which types and functions? Examples?

Exercise 5: Glycolysis Model

Goal

- Formulate and implement an abstract representation of *E. coli* glycolysis, given its topology, parameters and initial conditions.
- Estimate model parameters using available experimental data.

Note: This is a simplified model.