cAMP-mediated Regulation of pH Homeostasis

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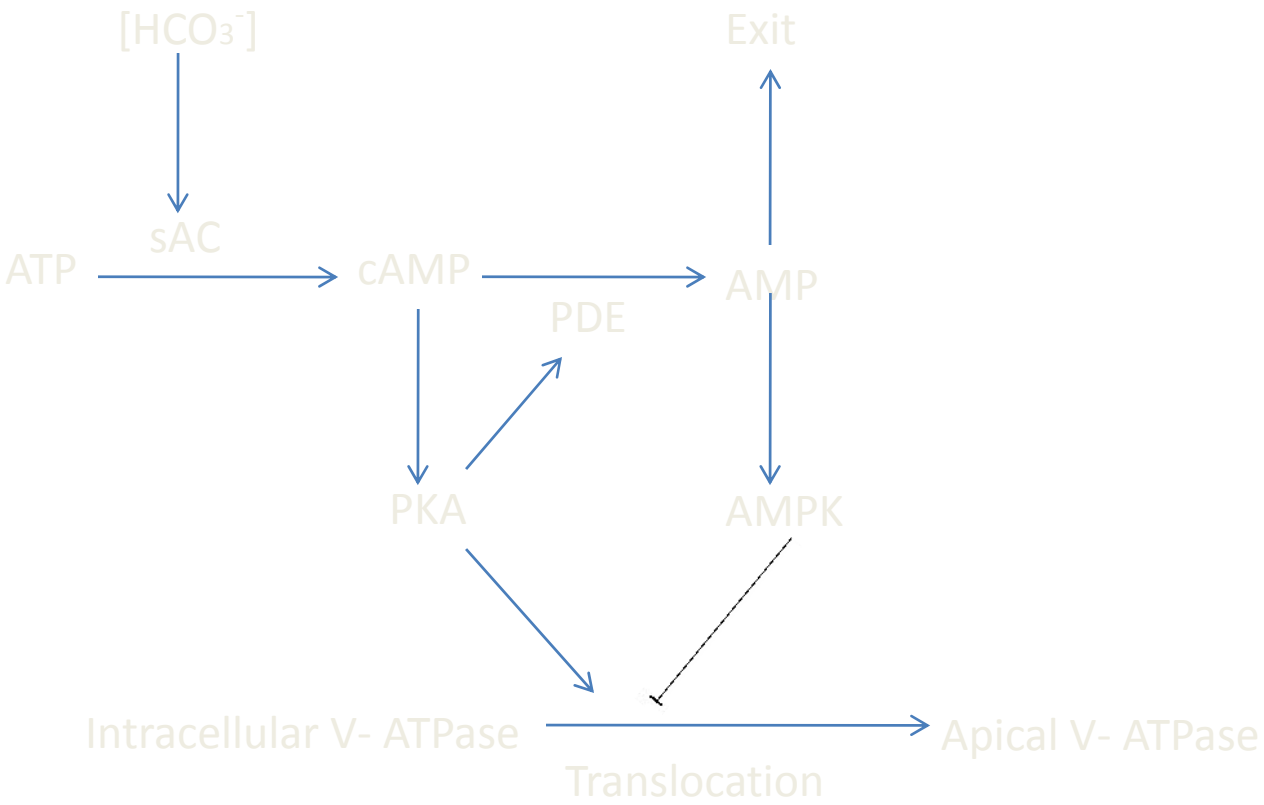
***Reproductive Biology, University of Pittsburgh

Quantitative Biology , June 3, 2009

Background/ Reaction Scheme



[HCO3-]
pH

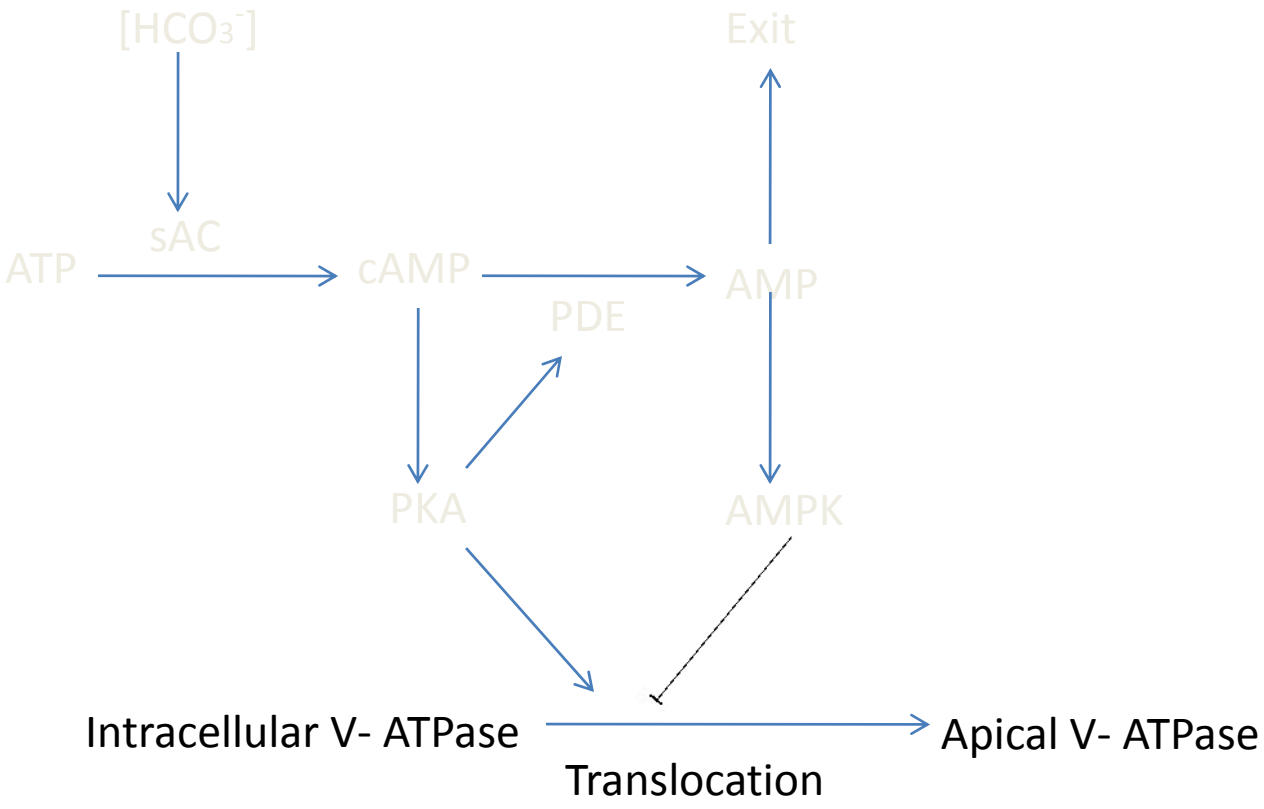
Normal pH=6.5
Low [HCO3-]

Cytoplasm

Lumen

Levine et al. (1978) . *J Reproduction and Fertility* 52:333-335
Breton S and Brown D (2007). *AJP-Renal Physiology* 292:F1-F10 (Review article)

Background/ Reaction Scheme



[HCO_3^-]
pH

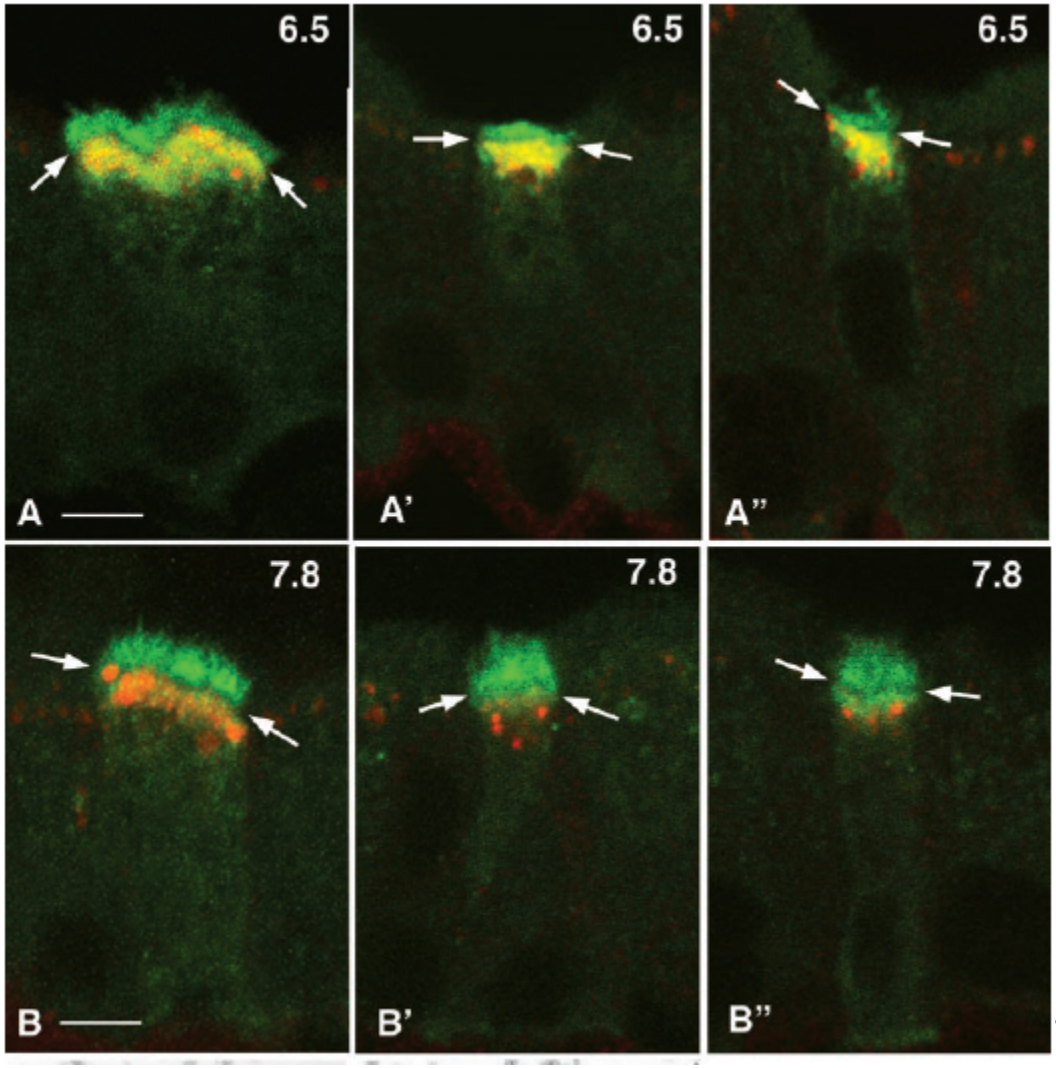
Normal pH=6.5
Low [HCO_3^-]

Breton S et al. (1996) *Nature Medicine* 2: 470-472
Brown D et al. (1996) *J Exp Biol* 199:2345-2358
Breton S et al. (2000) *Am. J. Physiol. Renal Physiol*. 278:F717-725

Increased Luminal pH results in increased V-ATPase surface expression

pH 6.5

pH 7.8

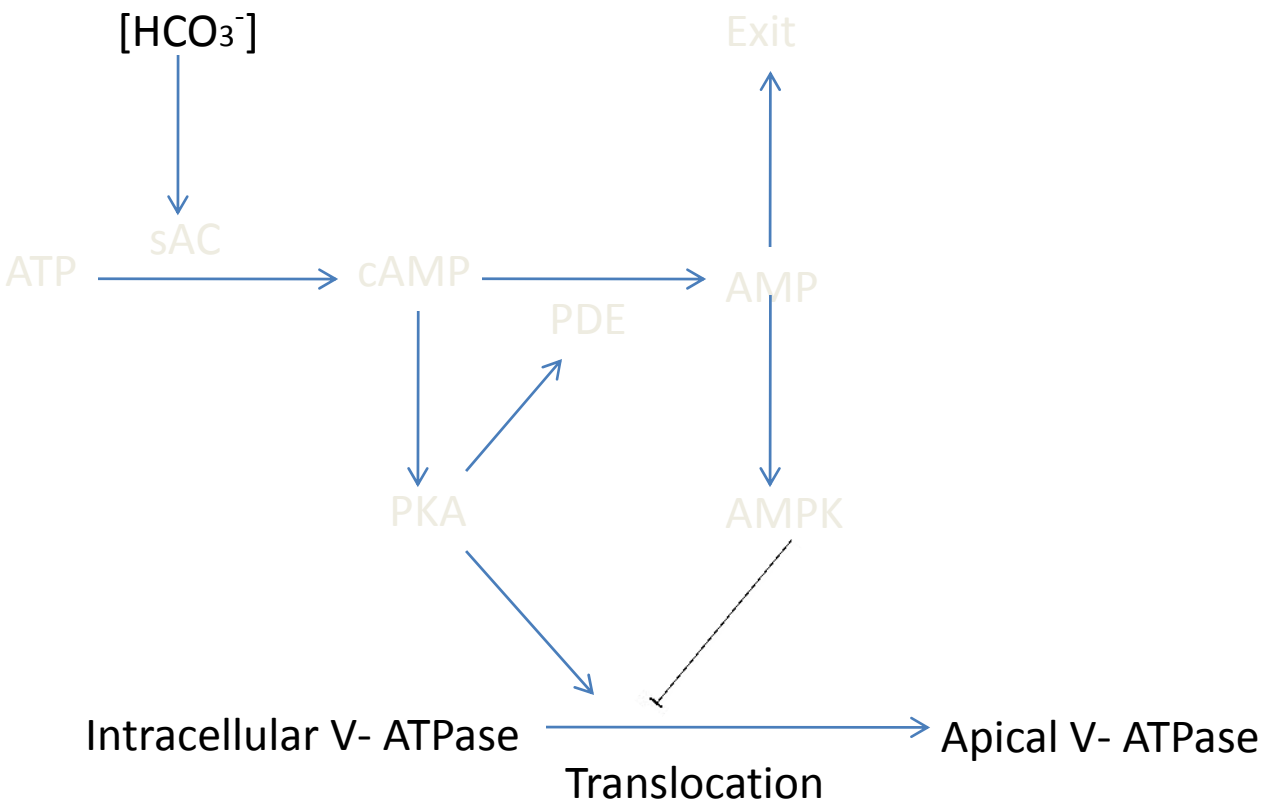


Scale bar = 5 micron

Confocal Images of Double Immunoflourescence labeling for V-ATPase (Green) and Endosomes (Red). Colocalization of V-ATPase and Endosomes (yellow) indicate intracellular location of the V-ATPase. The arrows indicate the frontier between the apical microvilli and subapical region.

Pastor-Soler et al. (2003) *JBC* 278 (49): 49523-49529

Background/ Reaction Scheme



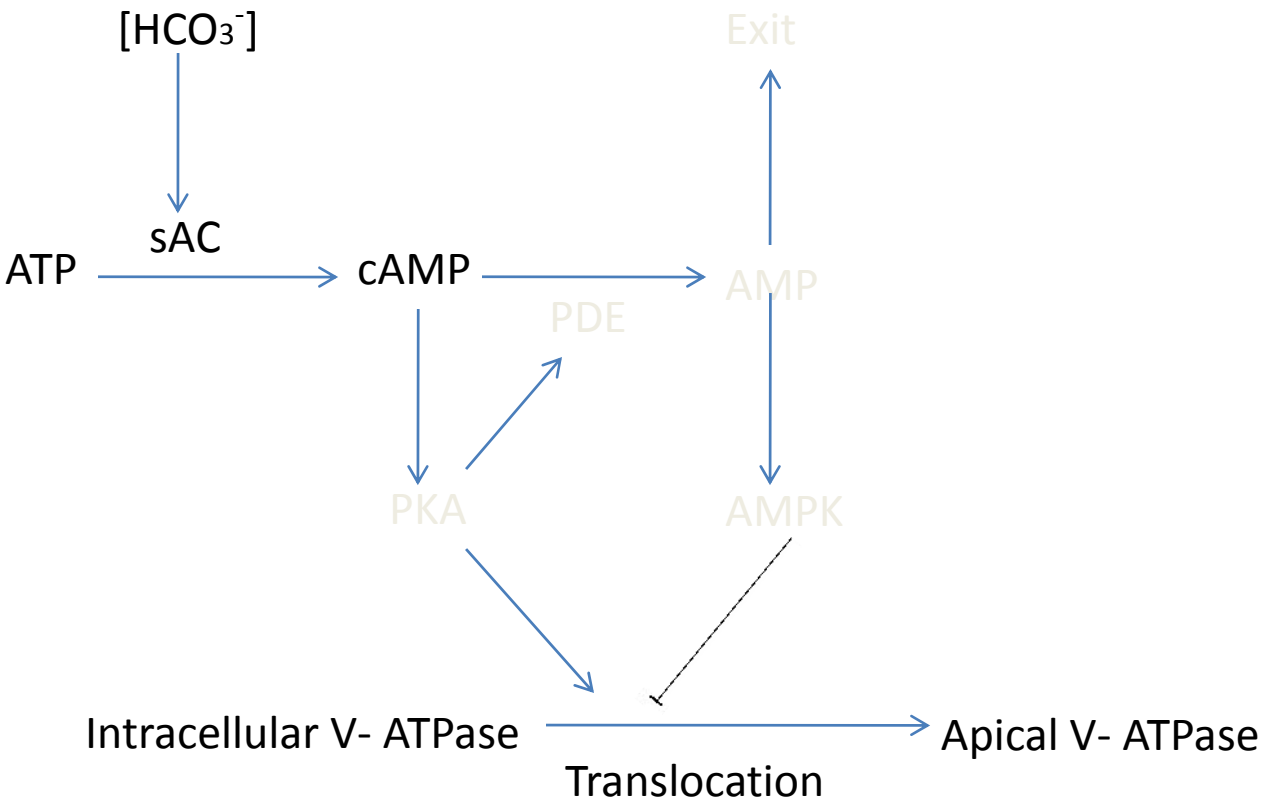
[HCO3-]
pH

Normal pH=6.5
Low [HCO3-]

Lumen

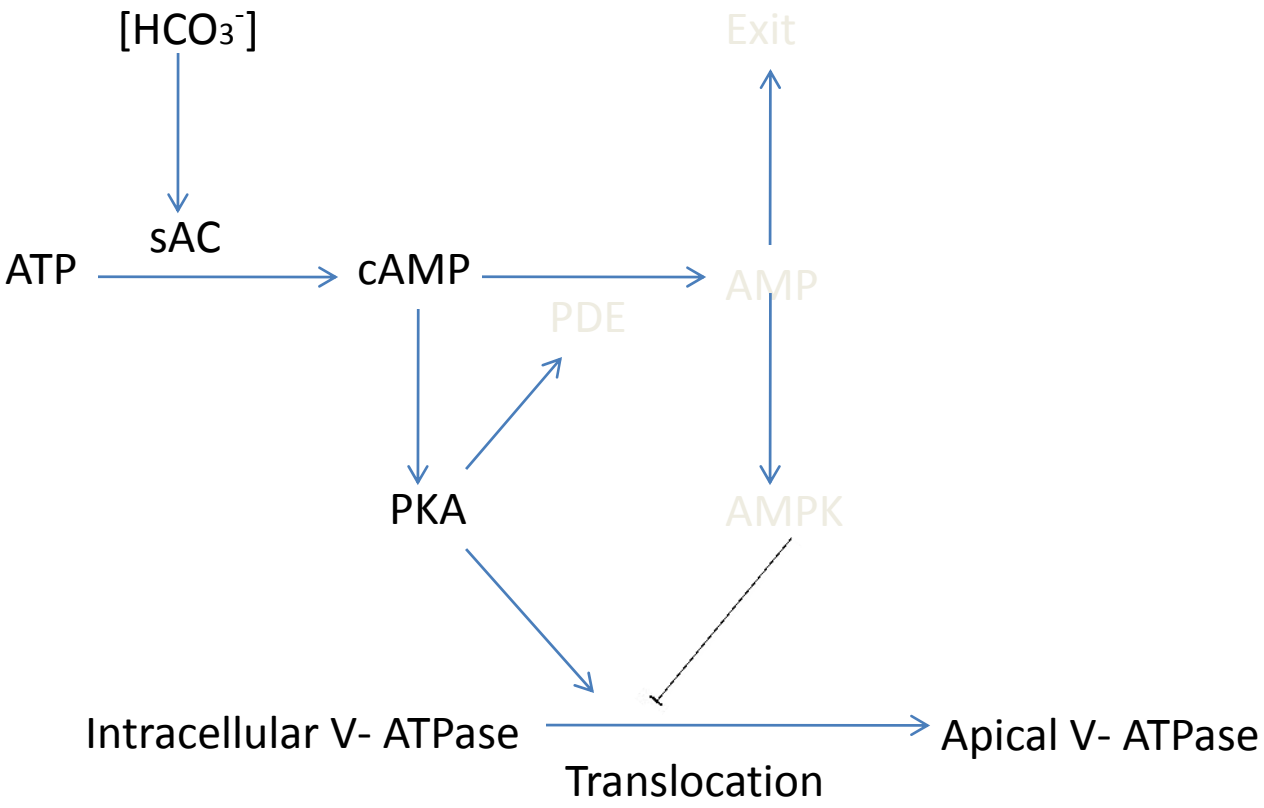
Breton S et al. (1998) Am. J. Physiol. Renal Physiol. 275: C1134-1142

Background/ Reaction Scheme



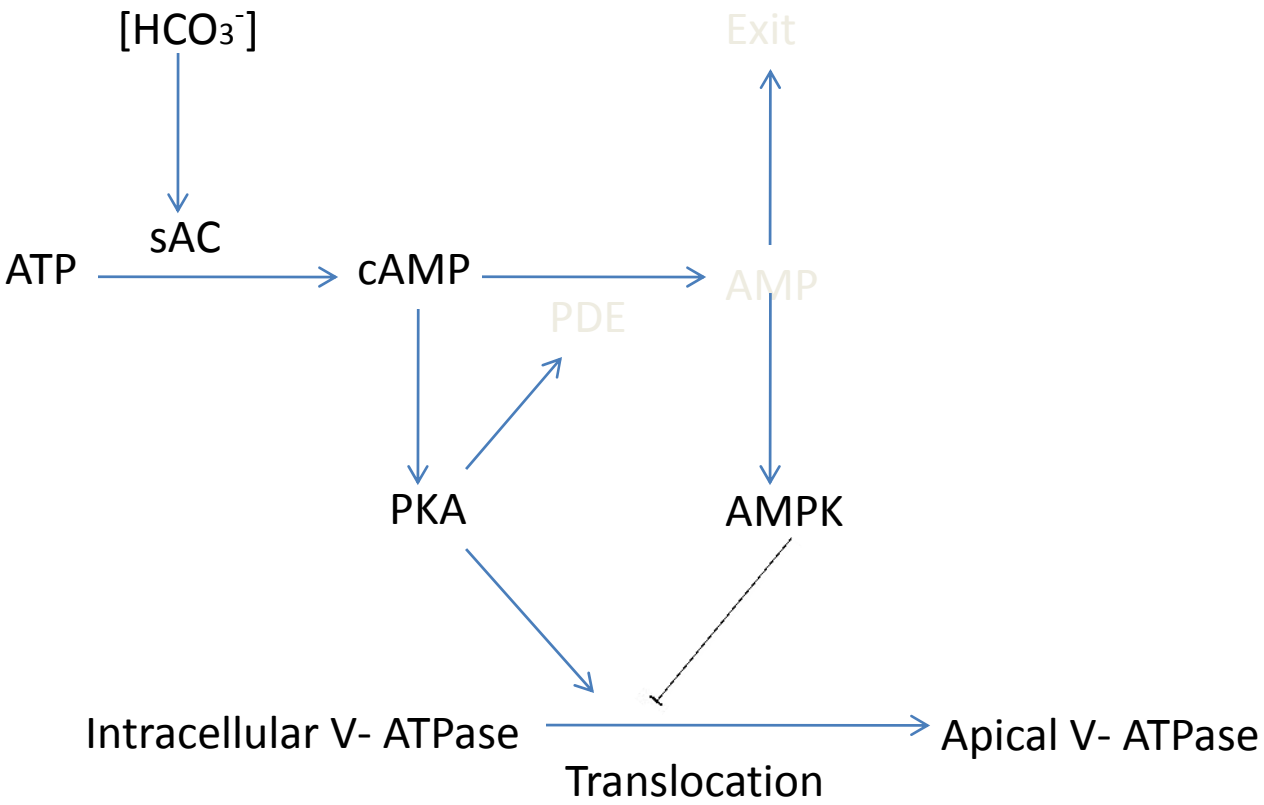
Chen et al. (2000). *Science* 289:625-628 (Levin/ Buck lab)
Pastor-Soler N et al. (2003) *JBC* 278 (49): 49523-49529

Background/ Reaction Scheme



Pastor-Soler N et al. (2008). *Am J Physiol Cell Physiol*. 294:488-494

Background/ Reaction Scheme



Tuerk RD et al. (2007). *J Proteome Res*. 6: 3266-3277

Hallows K et al. (2009). *Am J Physiol Cell Physiol*. 296: C672-C681

Hypothesis

The increase in [cAMP] that activates the V-ATPase translocation also stops it by activating AMPK through an increase in [AMP] due to cAMP degradation. (Prof. Lonny Levin's idea)

In this way, the sAC-cAMP-PKA pathway contains an inbuilt switch to automatically turn off the translocation process it started.

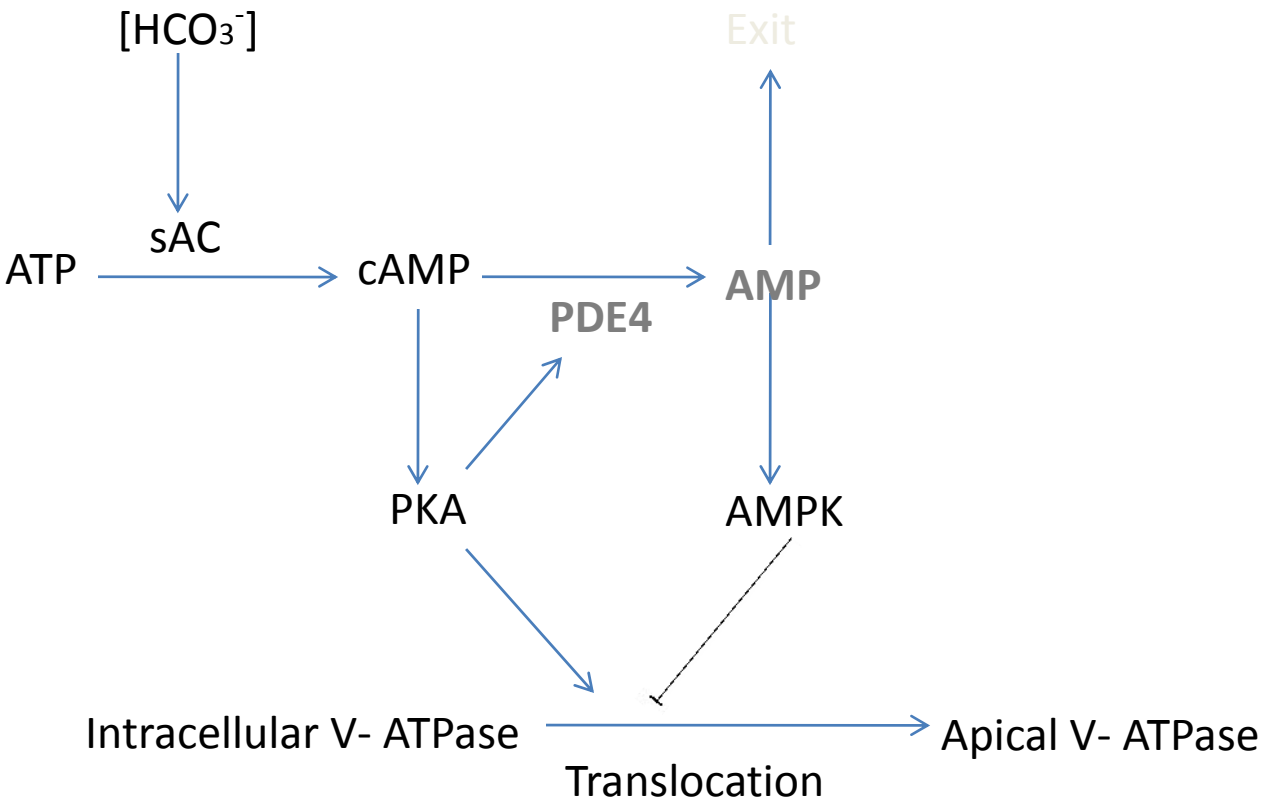
Model Objectives

Is our hypothesis plausible?

If yes, use the model to gain insights into the details of how activation of the bicarbonate-sAC-cAMP pathway starts and stops the V-ATPase translocation.

If yes, use the model to guide experiments.

Reaction Scheme

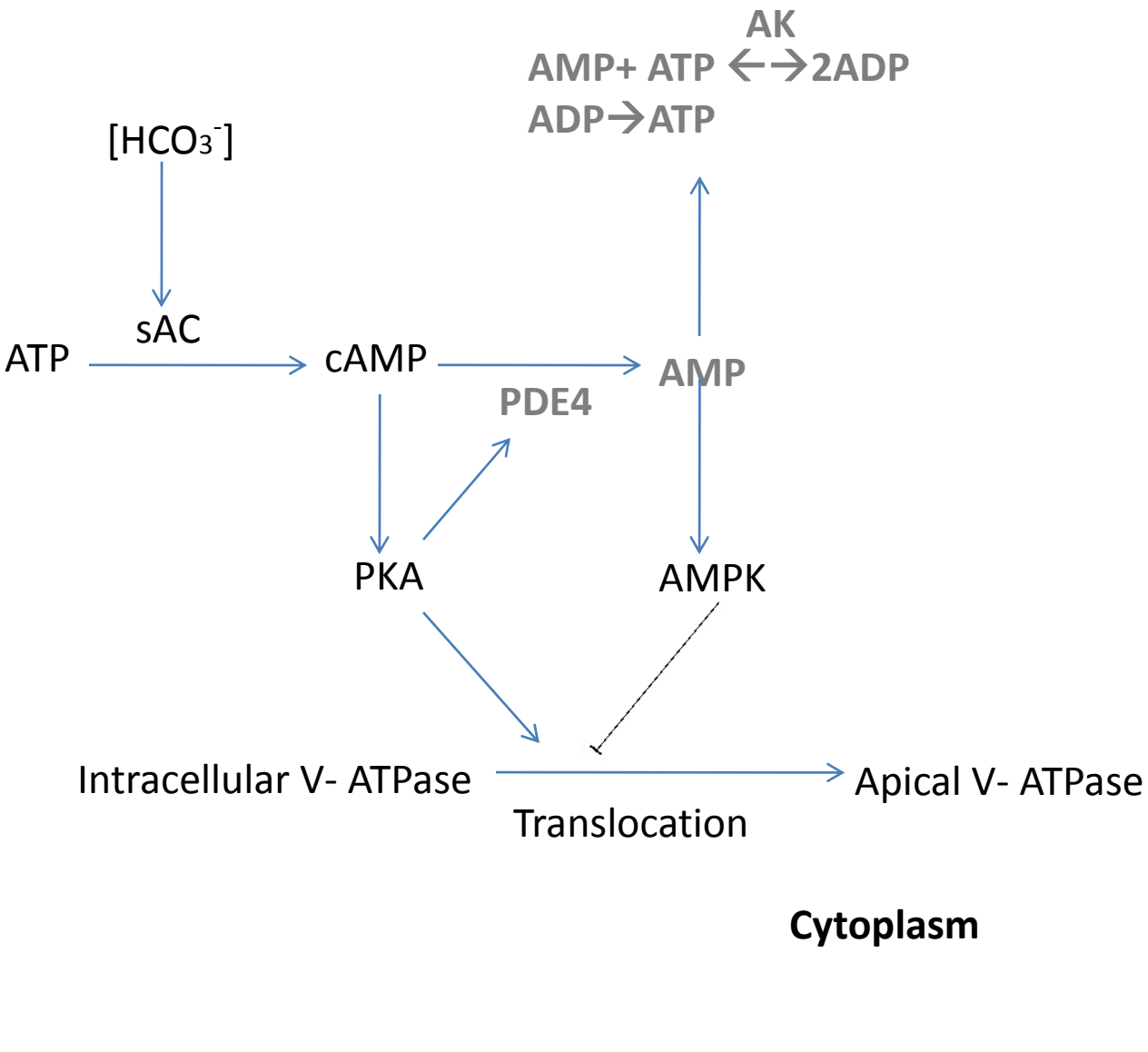


[HCO3-]
pH

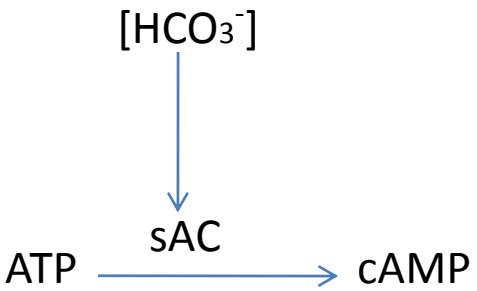
Normal pH=6.5
Low [HCO3-]

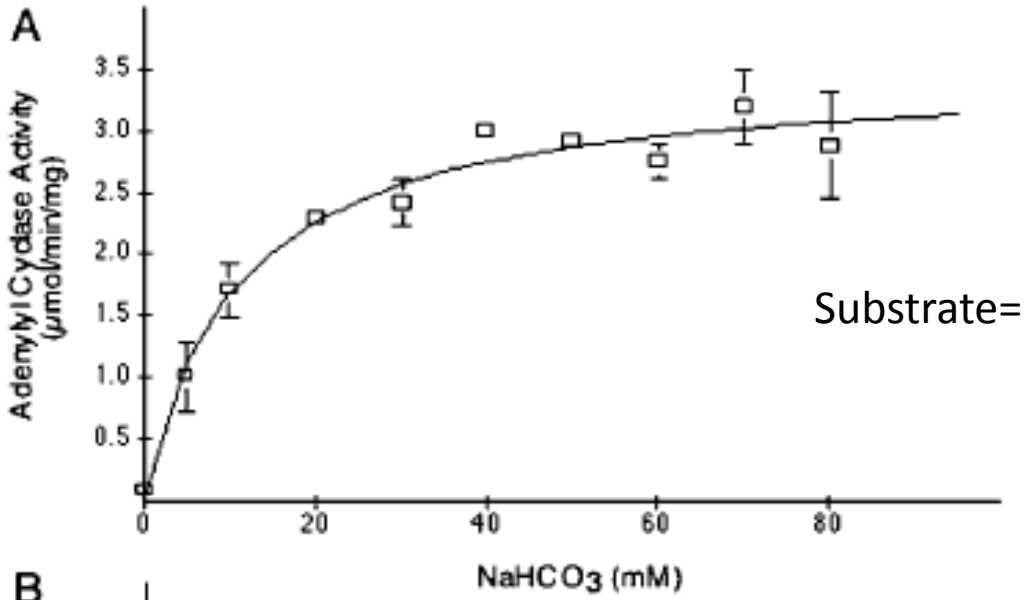
Lumen

Reaction Scheme

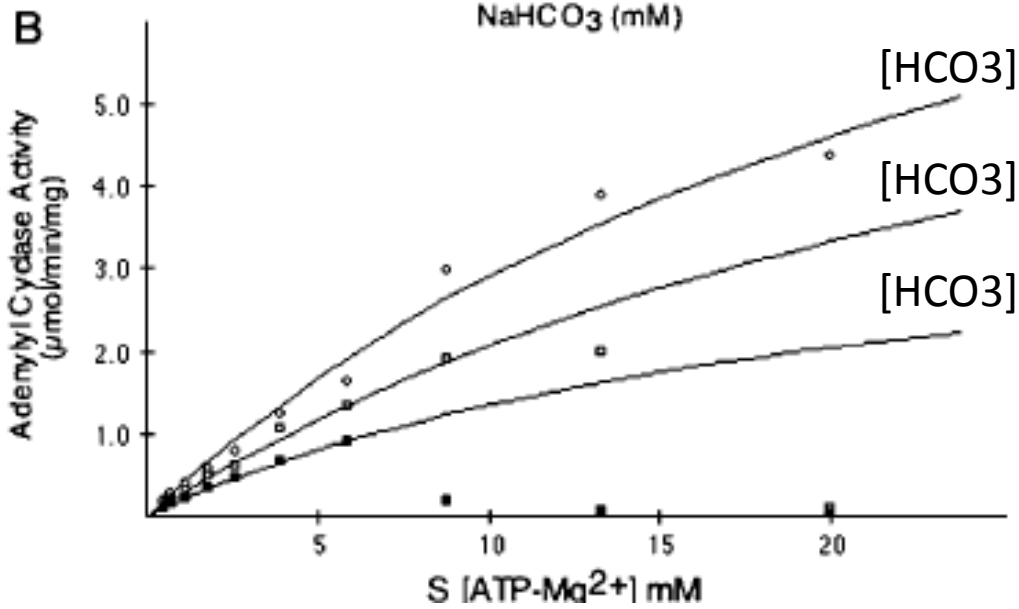


Model Parameters





Substrate=10 mM ATP



[HCO3]=50 mM

[HCO3]=15 mM

[HCO3]=0 mM

Michaelis – Menten

$$\text{Rate} = \frac{V_{\max} * [A]}{K_m + [A]}$$

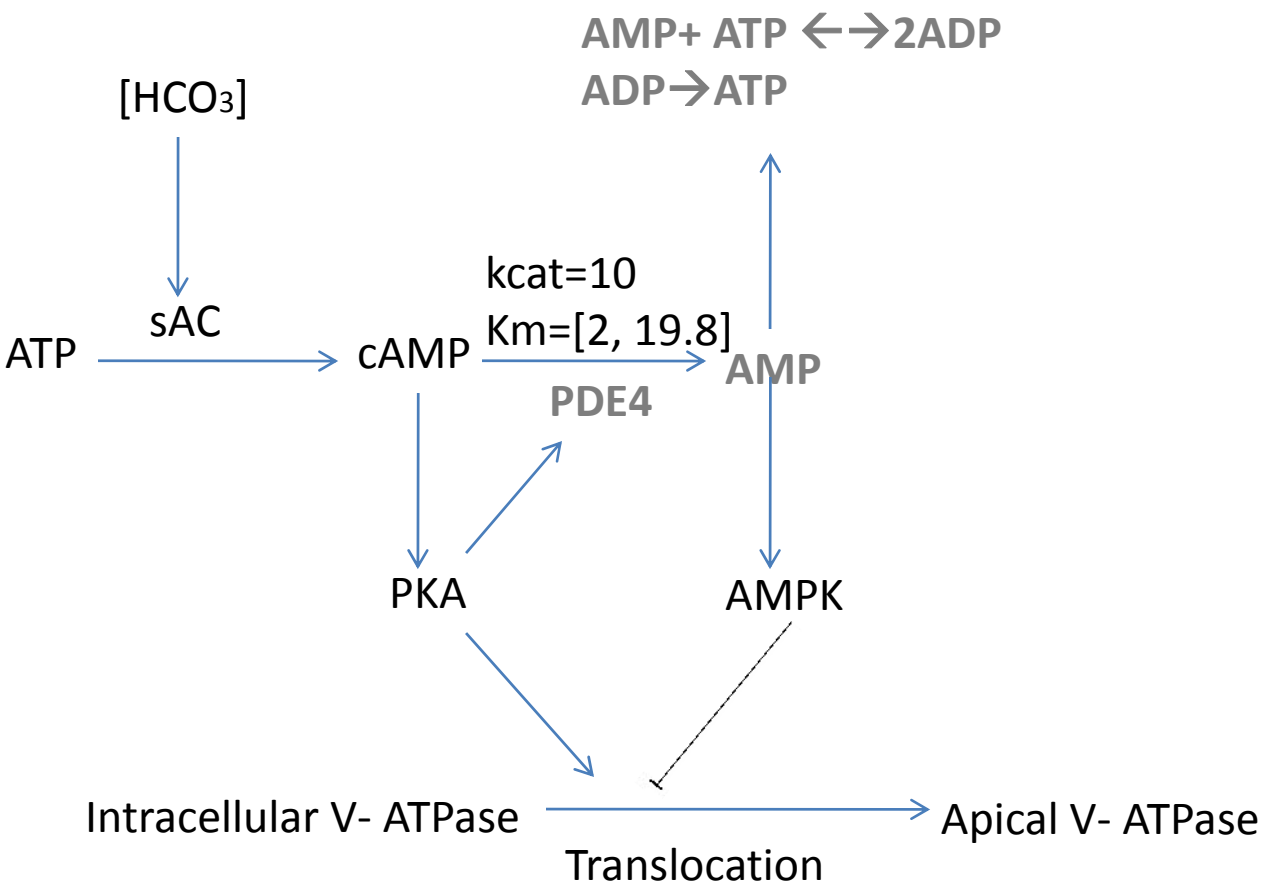
K_m= 10 mM

V_{max}=[1.6, 3.2] uMs-1

Litvin TN et al. (2003). *JBC* 278(18):15922-15926

Model Parameters

Units:
K_m: uM
K_{cat}: s-1



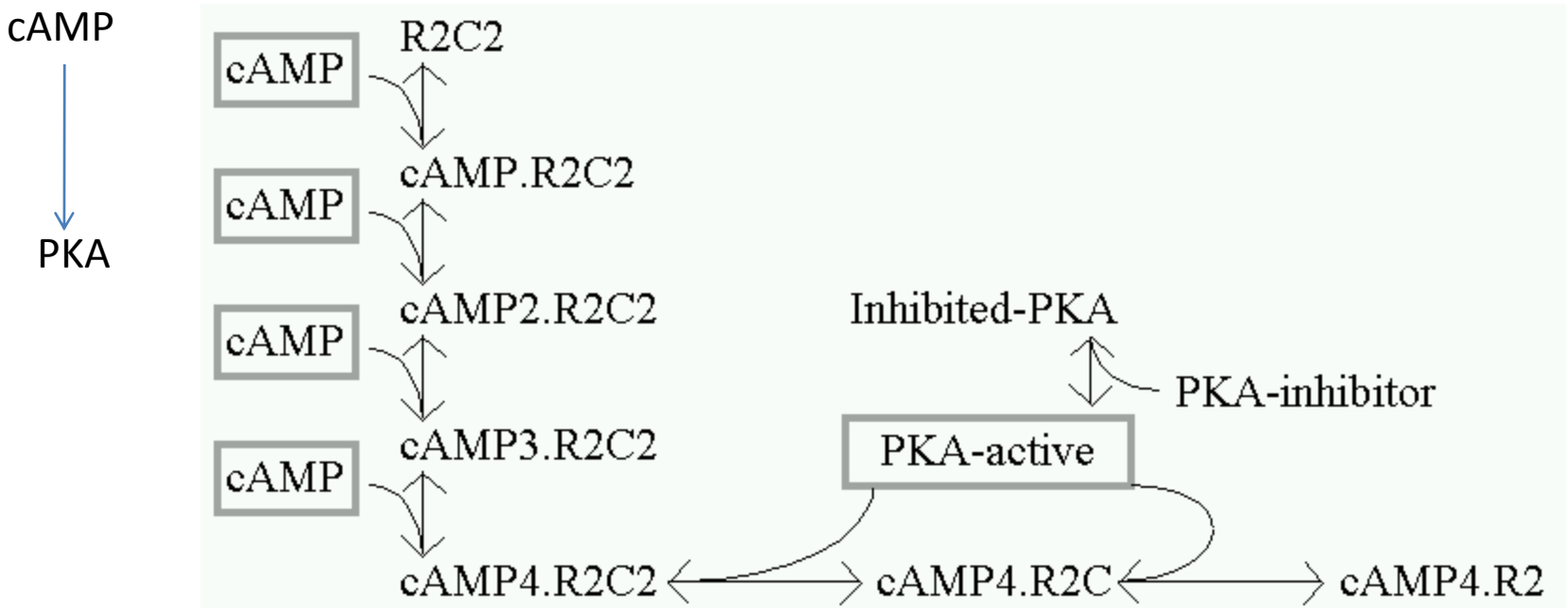
[HCO_3^-]
pH

Normal pH=6.5
Low [HCO_3^-]

Lumen

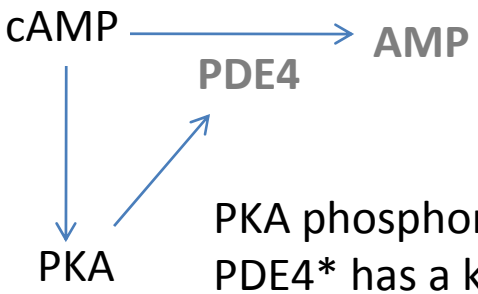
Bhalla US and Iyengar R (1999). *Science* 283:381-385
Bhalla US (NCBI) and Iyengar R (Mount Sinai), Database of Quantitative Cell Signaling
Conti M et al. (1995). *Biochemistry* 34:7979-7987

Model Parameters



Bhalla US and Iyengar R (1999). *Science* 283:381-385
Bhalla US (NCBI) and Iyengar R (Mount Sinai), Database of Quantitative Cell Signaling

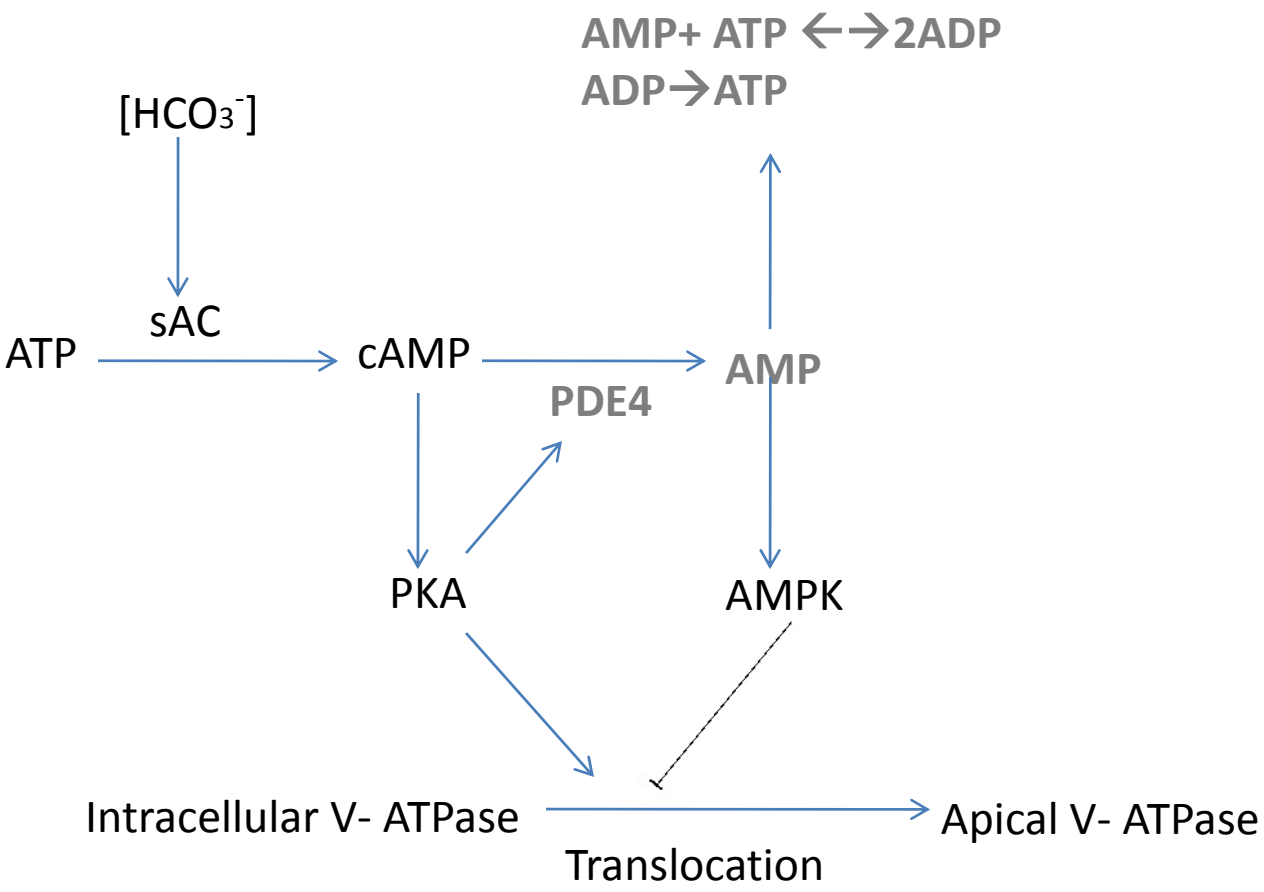
Model Parameters



PKA phosphorylates long PDE4 to a more active form PDE4*
PDE4* has a kcat of 20 and the same Km as PDE4

Bhalla and Iyengar, Database of Quantitative Cell Signaling
Mackenzie SJ (2002). *British Journal of Pharmacology* 136(3):421-433

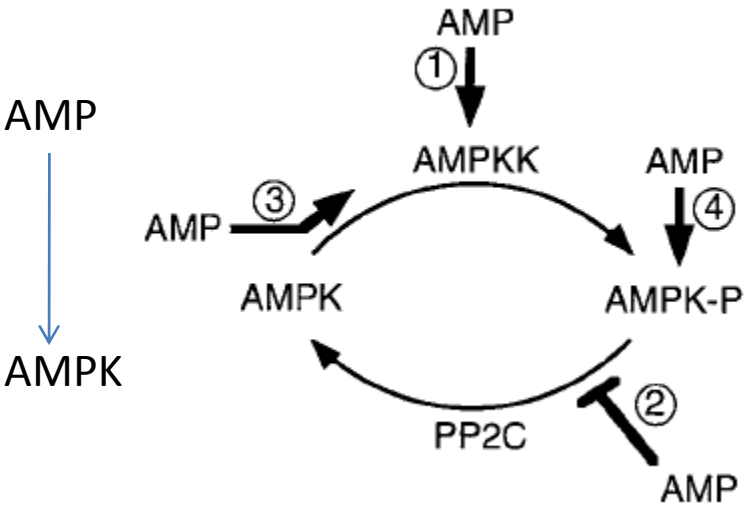
Model Parameters



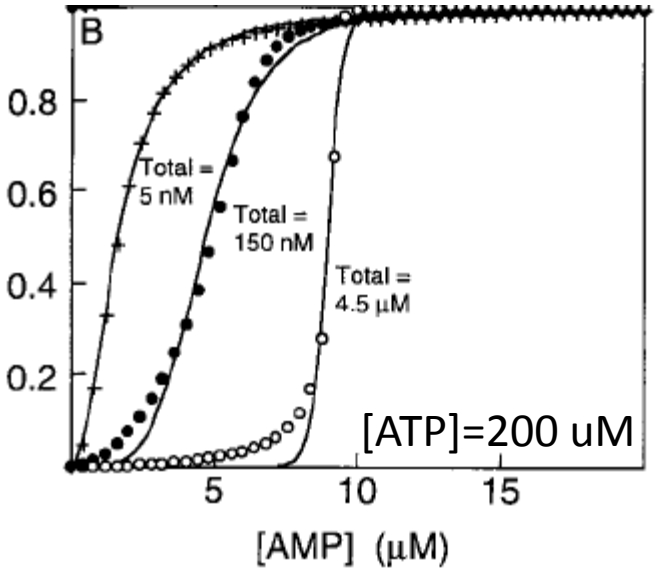
Model Parameters

AMP+ ATP ←→2ADP $K = \frac{[ATP]*[AMP]}{[ADP]*[ADP]} = 0.44$ kf~10 uMs-1

ADP→ATP Use constraint that a healthy cell typically maintains [ATP]:[ADP]:[AMP]=[100:10:1 ,100:20:1]

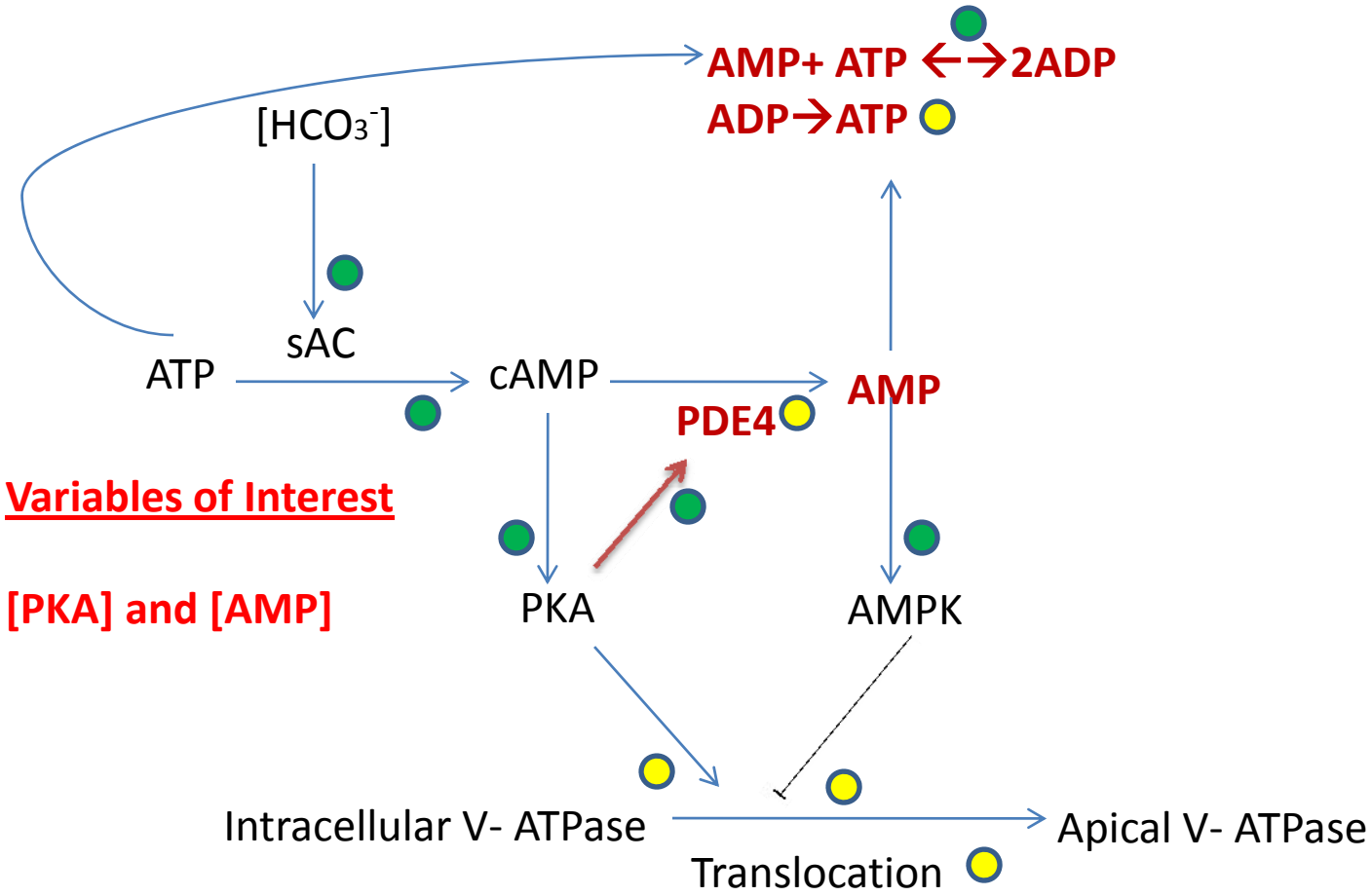


Fractional Activation of AMPK



Eggleston LV and Hems (1952). *Biochemical Journal* 52(1):156-160
Isidoris B and Newsholme EA (1975). *Biochemical Journal* 152:23-32
Hardie DG et al. (1999). *Biochemical Journal* 338:717-722
Hardie's reviews on AMPK as a metabolic sensor

Model



Stimulus
$[HCO_3^-]$ ↑
pH ↑

Normal
pH=6.5
Low $[HCO_3^-]$

The scheme contains assumed chemical species and reactions (maroon).
Green dot: parameters are known for the reaction.
Yellow dot: Parameters are unknown for the reaction.

Key Model Assumptions

In addition to the assumptions inherent in Michaelis-Menten/ massa action formalism,

1) PDE is PDE4, specifically long PDE4

2) The outlined reaction scheme can be isolated

1. Is our hypothesis plausible?

Equilibration Run

Initial condition

[ATP]=225 uM, [PDE]=0.5 uM, inactive [PKA]=0.5 uM, *
Initial concentrations of all other species = 0

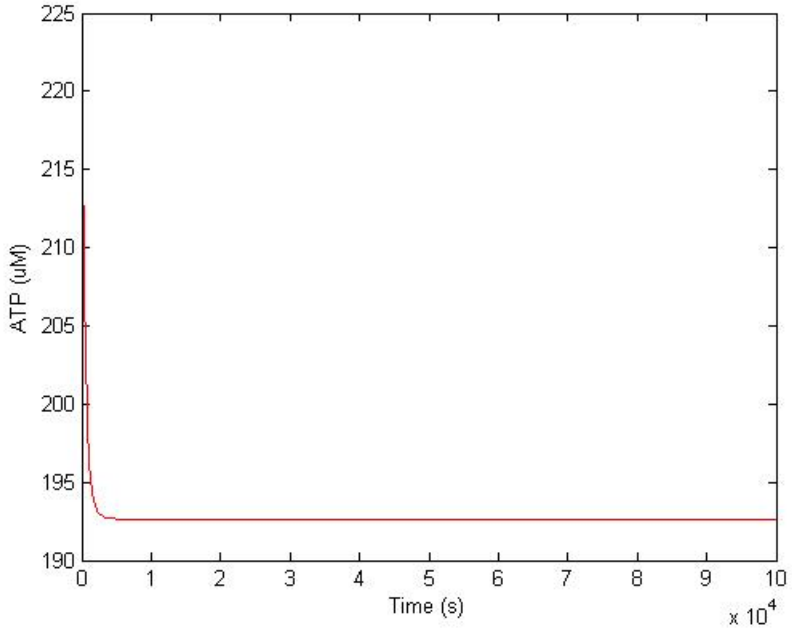
Starting from the initial conditions, the model is run till the system reaches equilibrium, which sets the modeled system to its basal values.

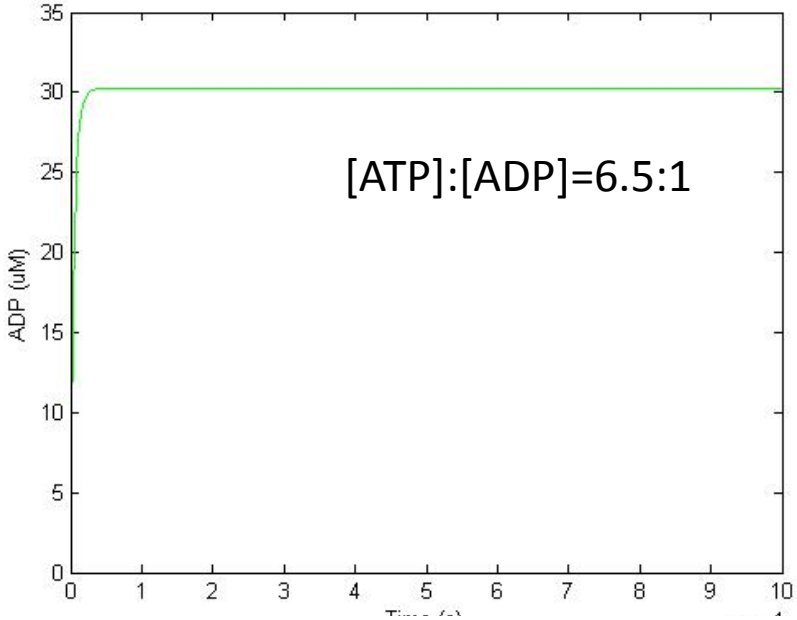
The model, if working properly, should equilibrate to physiological values for the concentration of the chemical species. Further, the concentrations should be in the regime that would be relevant to the function of interest.

* Bhalla and Iyengar, Database of Quantitative Cell Signaling

Equilibration run. Basal [AMP] is found to be at the lower end of the regime in which it impacts AMPK activation

[ATP] ~ 200 uM regime





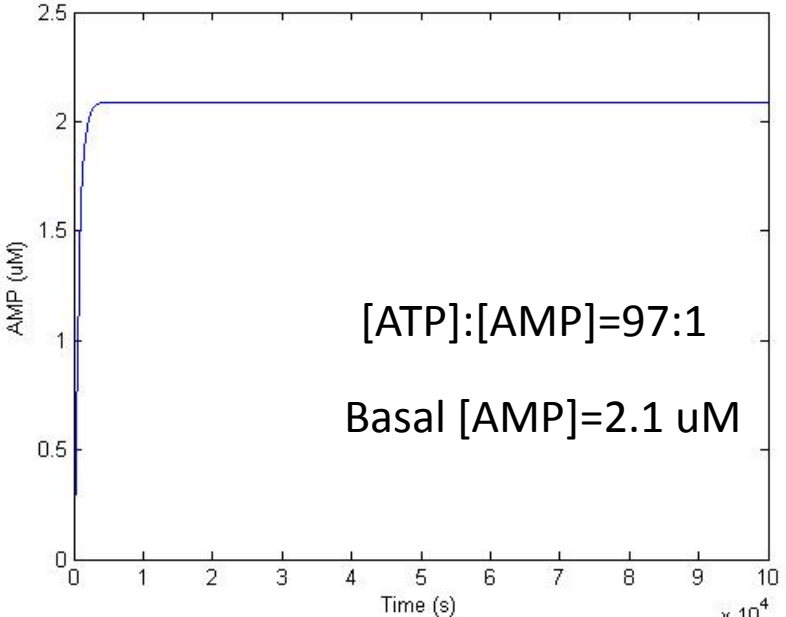
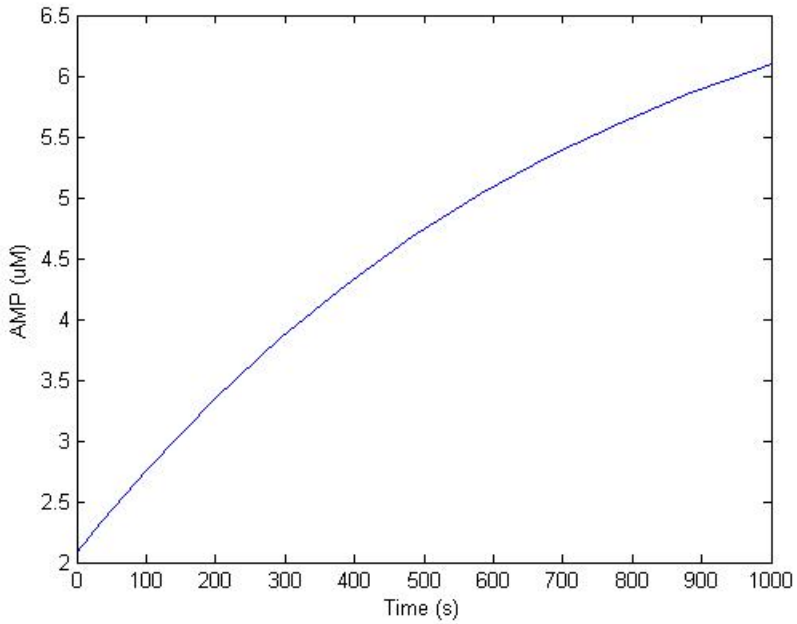


Table 1 Basal concentrations of the different chemical species at different situations. PDE* denotes PDE activated by PKA

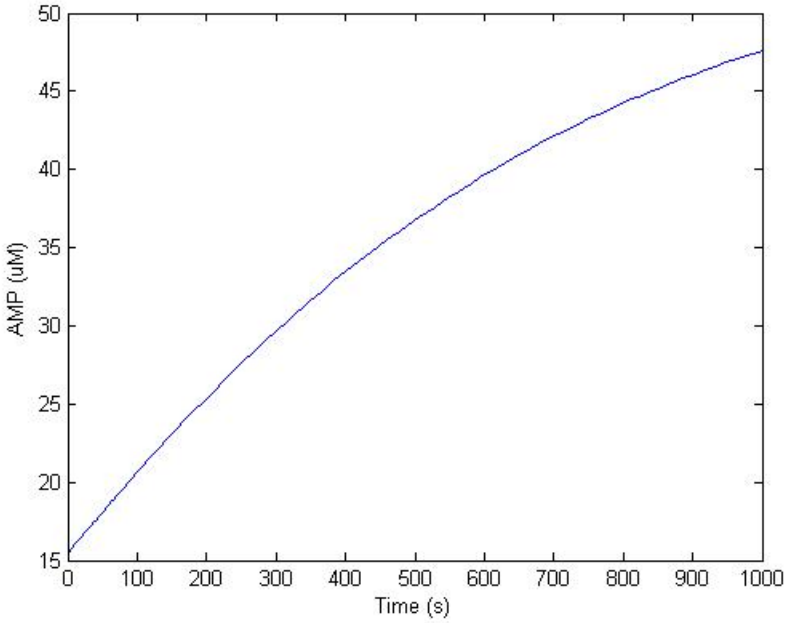
Situation	ATP (uM)	ADP (uM)	AMP (uM)	cAMP (uM)	Active PKA (uM)	PDE (uM)	PDE* (uM)
Normal Km for PDE =6 uM	193	30.2	2.1	0.032	0.0164	0.42	0.08

Sustained near-maximal bicarbonate stimulus increases [AMP] to the upper end of the [AMP] regime in which AMP can impact AMPK activation

The stimulus is modeled by a two-fold increase in V_{max} of sAC



[ATP]~ 200 uM regime

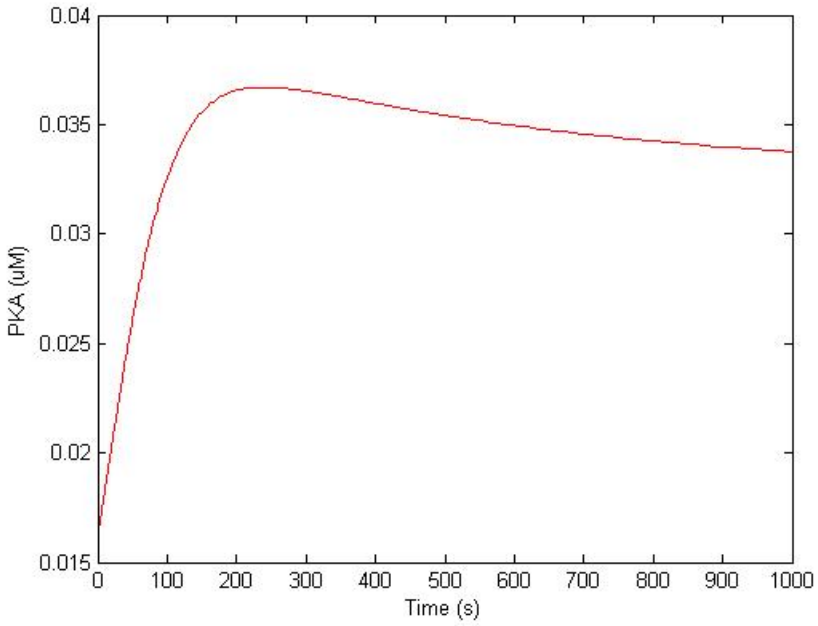


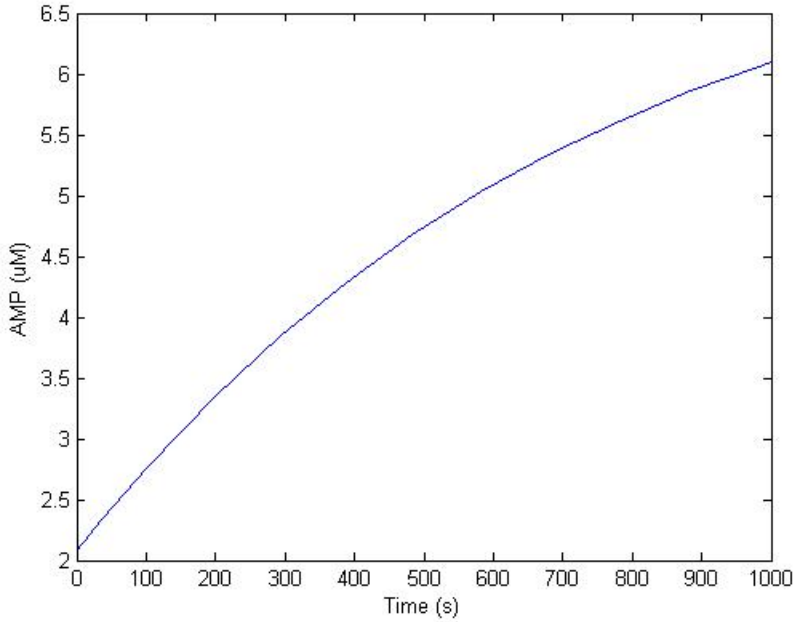
[ATP]~ 2000 uM regime

In this regime, [AMP] can impact AMPK activation till about [AMP]=50 uM, based on the Hill equation involved

From now on, I will show the figures for the [ATP]~ 200 uM regime only, but the conclusions apply to the 2000 uM regime as well.

In response to sustained bicarbonate stimulus, active [PKA] first rises and then [AMP], thus giving a time window in which V-ATPases are phosphorylated by PKA and can translocate





"PKA" in the figure axis refers to active PKA

In my model, the PKA remains high as long as the bicarbonate level is high. After the luminal pH and the intracellular bicarbonate concentration goes down back to its basal level, the system will reset to its basal level.

Table 2 Time window for V-ATPase translocation towards the apical membrane vs. Increase in bicarbonate concentration. The time window is found to increase with increasing bicarbonate concentration, allowing V-ATPase translocation to occur for a longer time.

$V_{max}/V_{max,basal}$ Of sAC	PKA peak (uM)	Time to active [PKA] peak (s)	[AMP] maximal response (uM)	Time to AMP half-maximal (s)	Time Window Indicator (s)
1.2	0.02	700	2.9	400	402-700= **-300**
1.4	0.024	540	3.8	410	410-540= **-130**
1.6	0.028	410	4.9	437	437-410 =**27**
1.8	0.032	300	6	450	450- 300 =**150**
2	0.036	225	7.2	452	452-225 =**227**

So far…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

So far…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

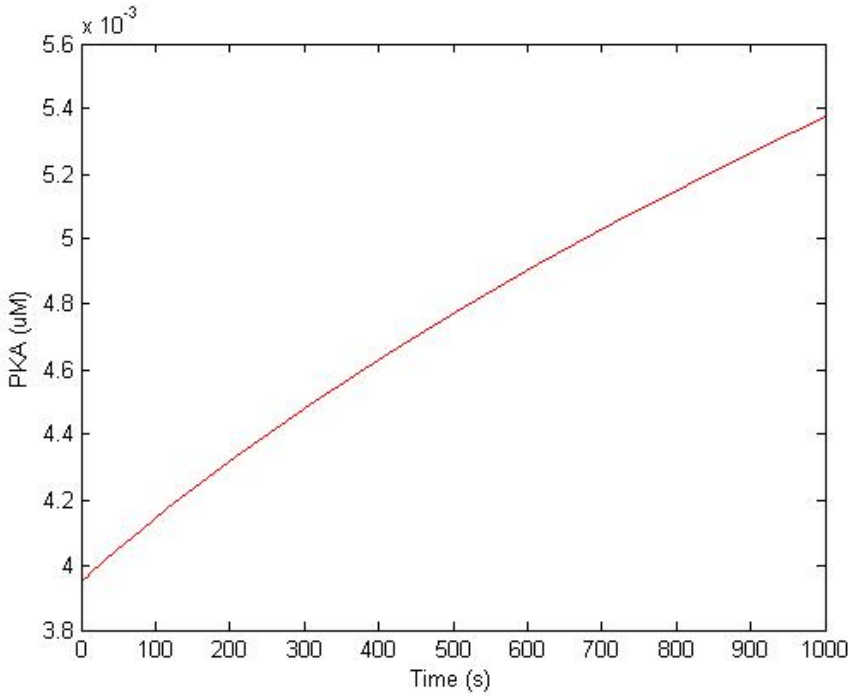
Next…

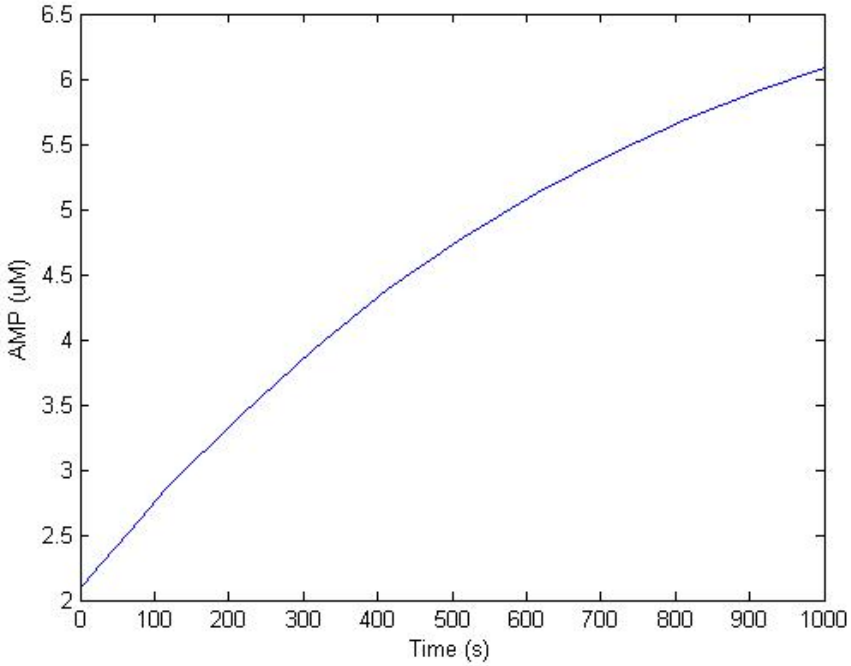
Is our conclusion robust to the choice of PDE isoform?

Table 1 (again) Basal concentrations of the different chemical species at different situations. PDE* denotes PDE activated by PKA

Situation	ATP (uM)	ADP (uM)	AMP (uM)	cAMP (uM)	Active PKA (uM)	PDE (uM)	PDE* (uM)
Normal Km for PDE =6 uM	193	30.2	2.1	0.032	0.0164	0.42	0.08
Km for PDE =2uM	193	30.2	2.1	0.012	0.0039	0.48	0.02
Km for PDE =19.84uM	193	30.2	2.1	0.083	0.0715	0.27	0.23

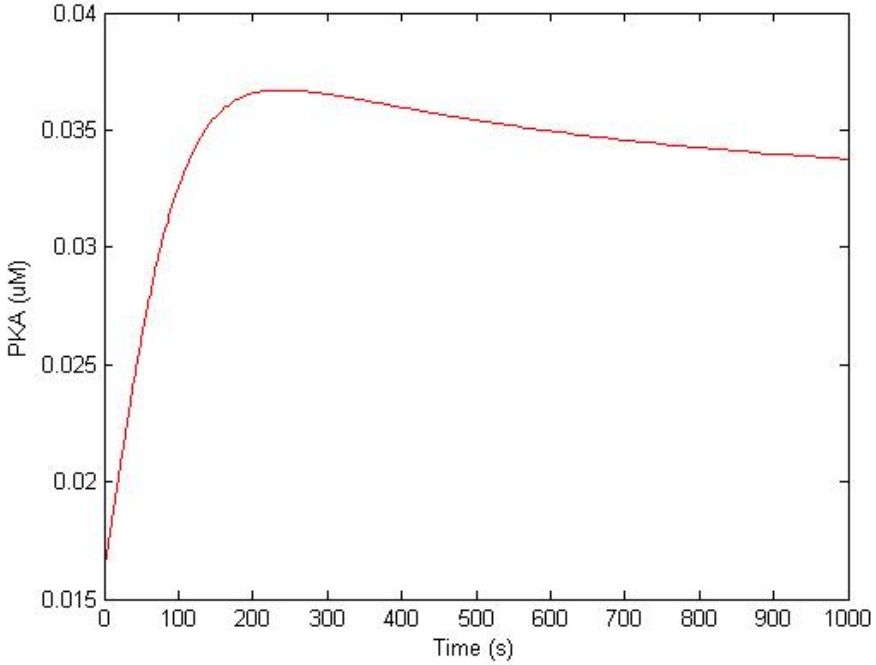
Response to bicarbonate stimulus

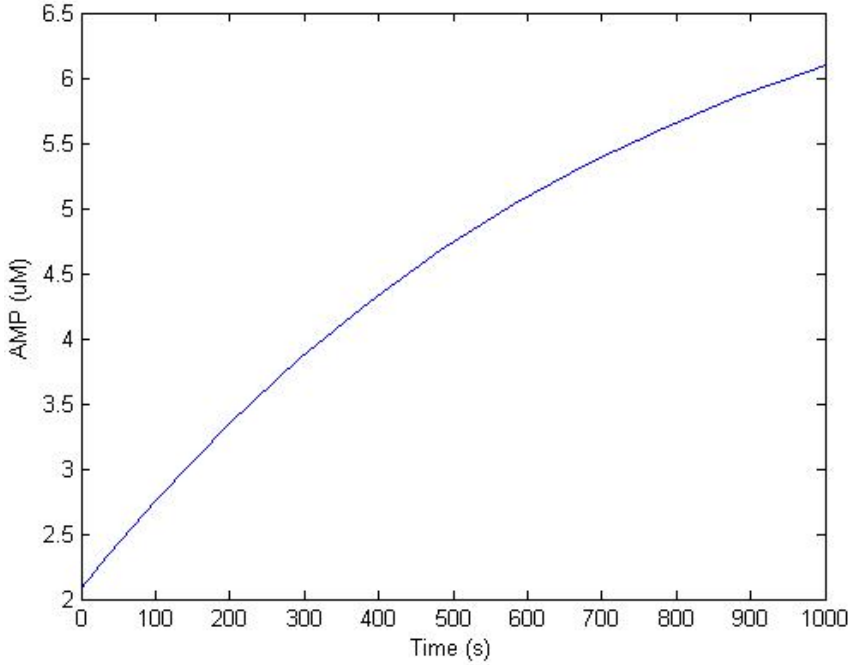




K_m for cAMP-PDE= 2 uM

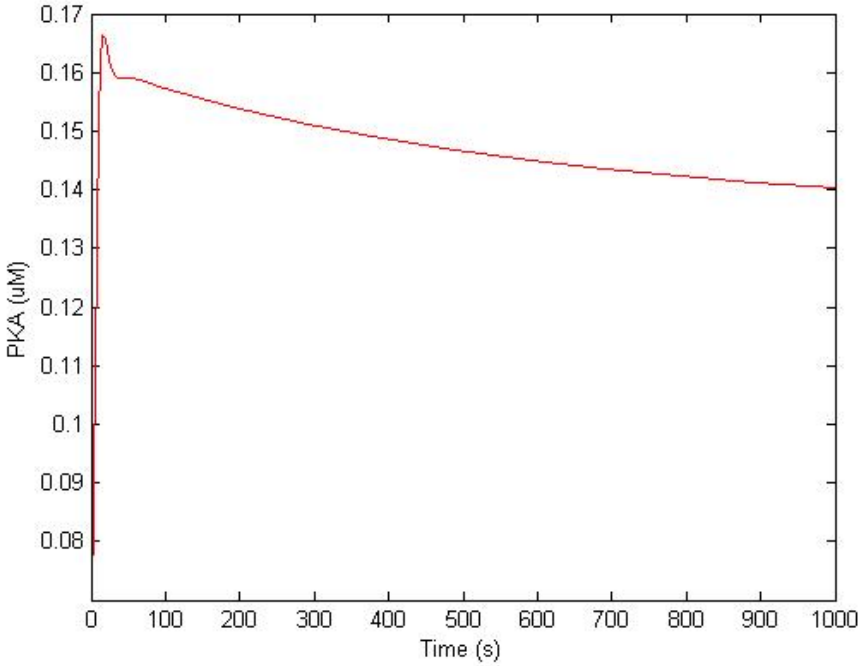
Response to bicarbonate stimulus

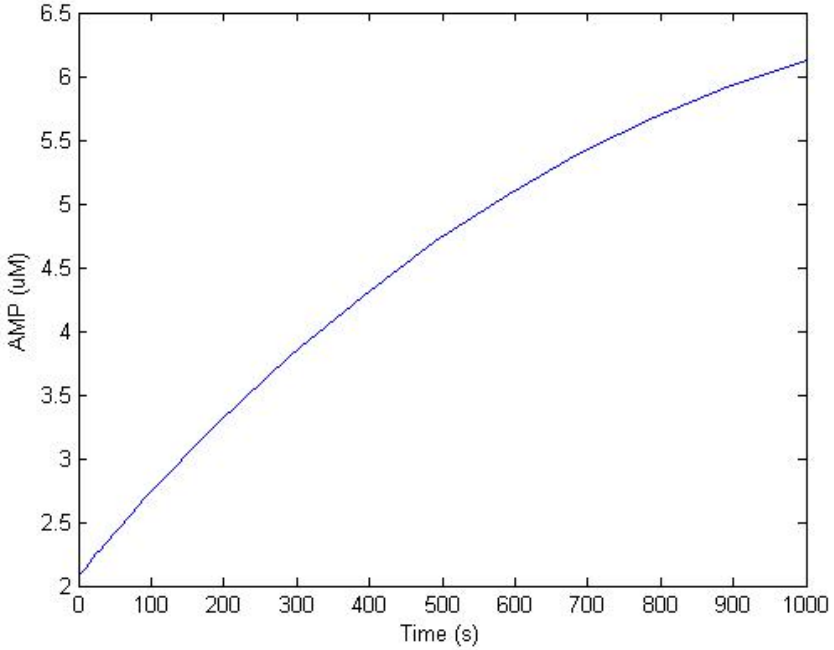




K_m for cAMP-PDE= 6 uM

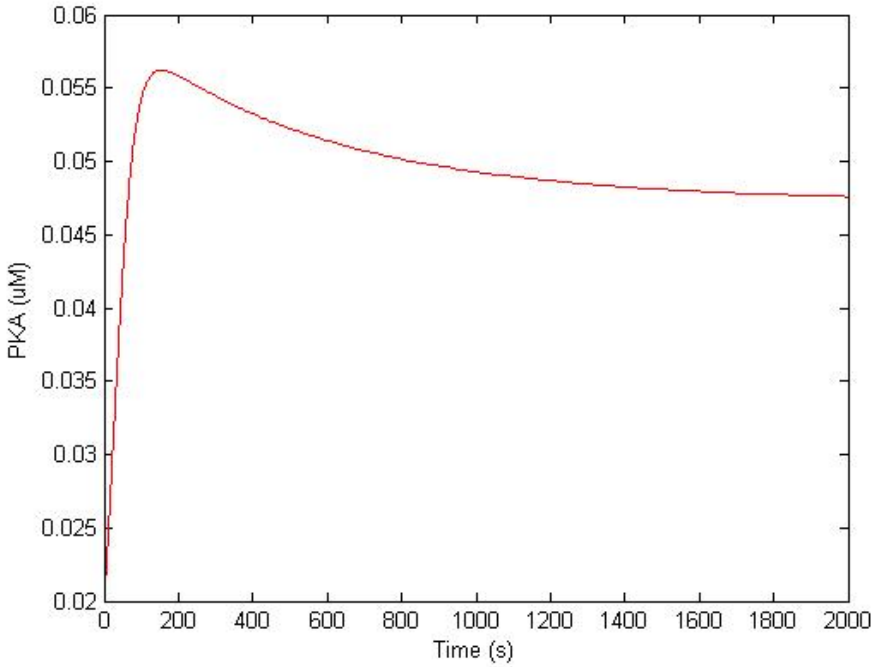
Response to bicarbonate stimulus

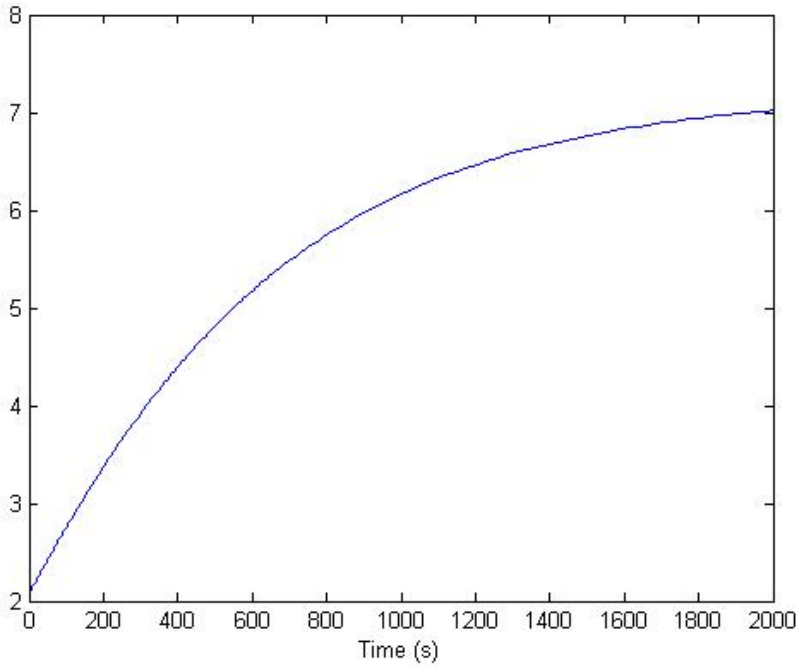




K_m for cAMP-PDE= 19.84 uM

Response to bicarbonate stimulus





K_m for cAMP-PDE= 6 uM

PKA does not activate PDE*

Summary…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

Our conclusion is robust to the choice of PDE isoform in the model

Summary…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

Our conclusion is robust to the choice of PDE isoform in the model

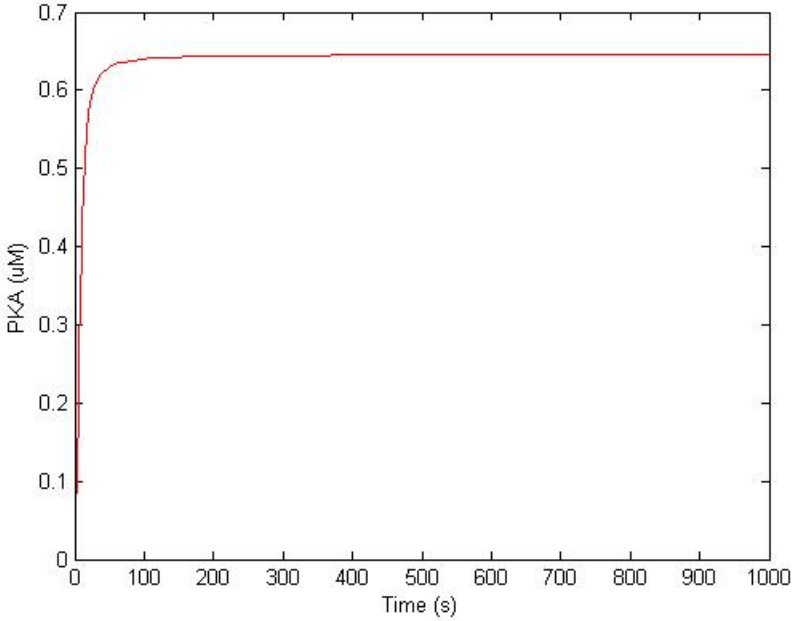
Next…

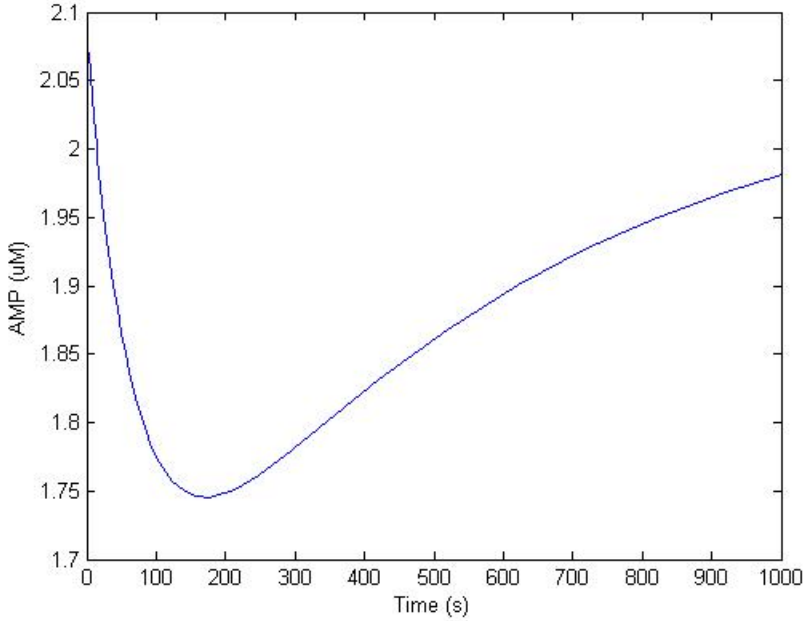
How shall we test the model boundary?

Testing the Model Boundary

Virtual Experiment:
At time 0, apply 10 uM of Rolipram-- a specific inhibitor of PDE4

Rolipram is modeled by an increase in the Km for the cAMP-PDE reaction as Km_inhibited=Km*(1+[rolipram]/KI), with a KI=0.09 uM

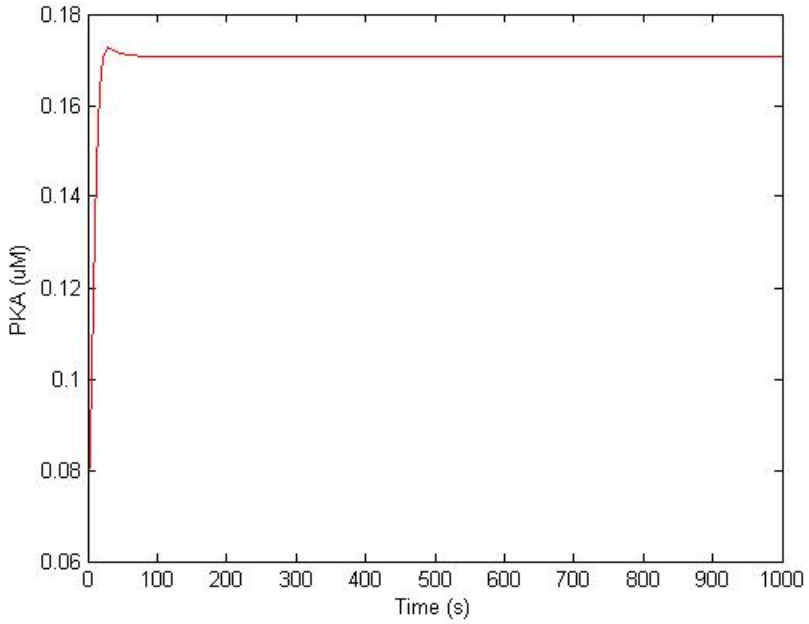


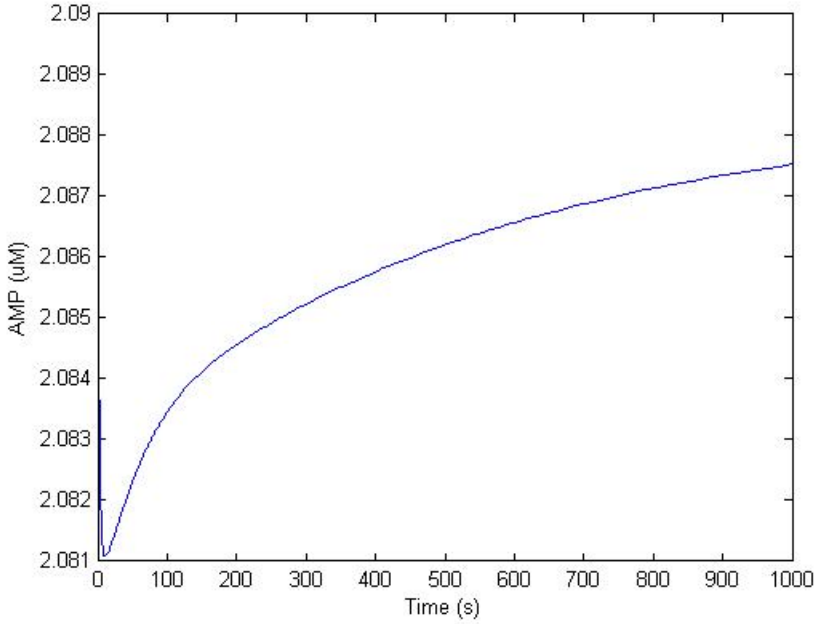


Saturating concentration of Rolipram activates PKA but does not increase [AMP] (actually, it results in a transient decrease in [AMP])

Testing the Model Boundary

Virtual Experiment:
At time 0, apply 0.1 uM of Rolipram-- a specific inhibitor of PDE4





The corresponding wet-lab experiment would allow us to determine the contributions of mechanisms that are absent in our model towards stopping the V-ATPase translocation

Summary…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

Our conclusion is robust to the choice of PDE isoform is the model

We have used the model to suggest a wet-lab experiment to test the model boundary and estimate contributions of other mechanisms towards stopping the V-ATPase translocation

Summary…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

Our conclusion is robust to the choice of PDE isoform is the model

We have used the model to suggest a wet-lab experiment to test the model boundary and estimate contributions of other mechanisms towards stopping the V-ATPase translocation

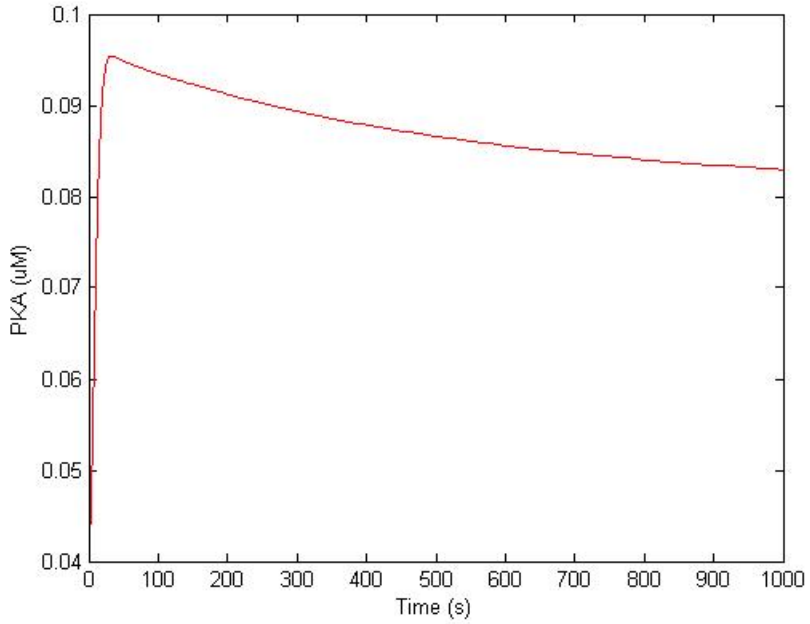
Next…

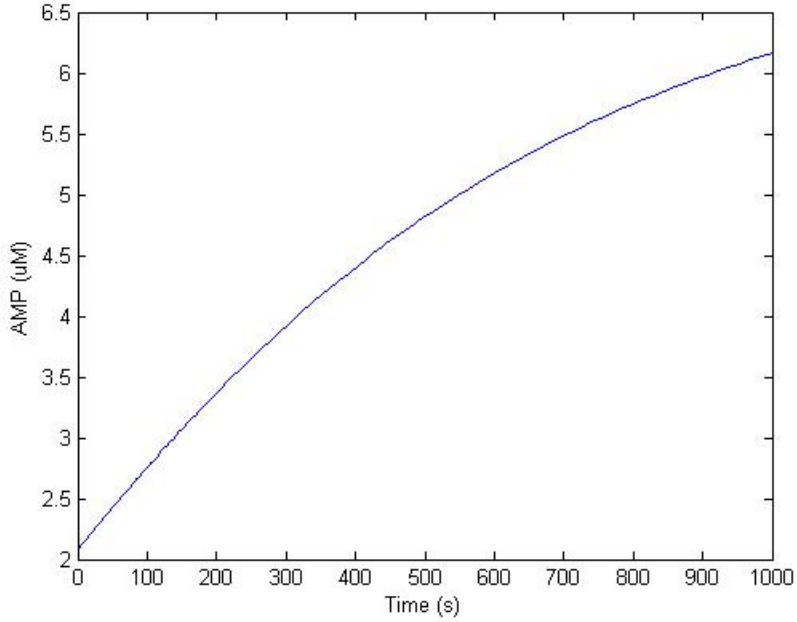
Use the model to understand the AMP response.

Bicarbonate-induced response of system pre-perfused with inhibitor of PDE

Virtual Experiment:

The system is pre-perfused with Rolipram (i.e., the system is allowed to equilibrate under treatment of Rolipram), after which the near-maximal bicarbonate stimulus is applied.



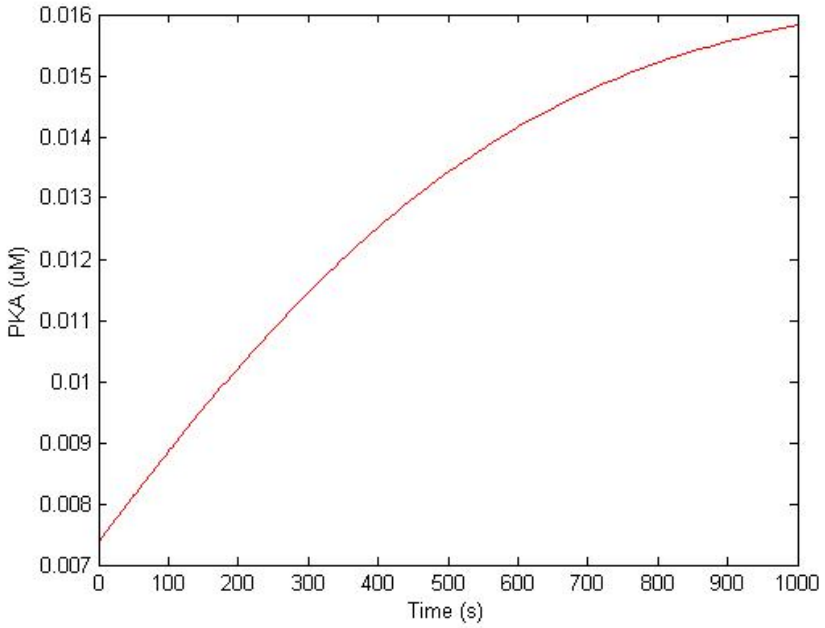


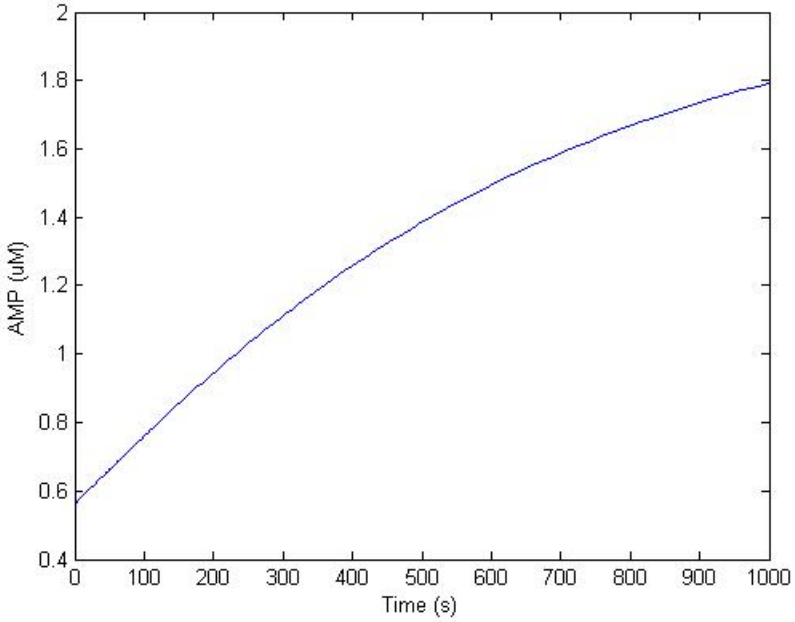
A strong PKA activation is observed, but the [AMP] rise is not impacted by the pre-perfusion with PDE inhibitor

Bicarbonate-induced response of system pre-perfused with inhibitor of sAC

Virtual Experiment:

The system is allowed to equilibrate with a 50% reduction in sAC V_{max} , after which the near-maximal bicarbonate stimulus is applied.





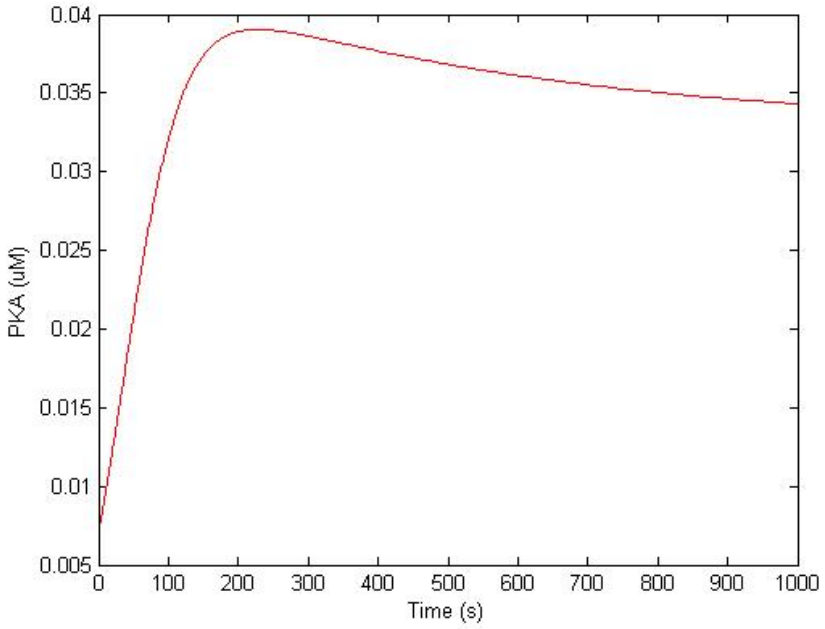
Response to a bicarbonate-induced two-fold increase in basal inhibited sAC V_{max}
Pre-perfusion with sAC inhibitor impairs AMP response to the bicarbonate stimulus

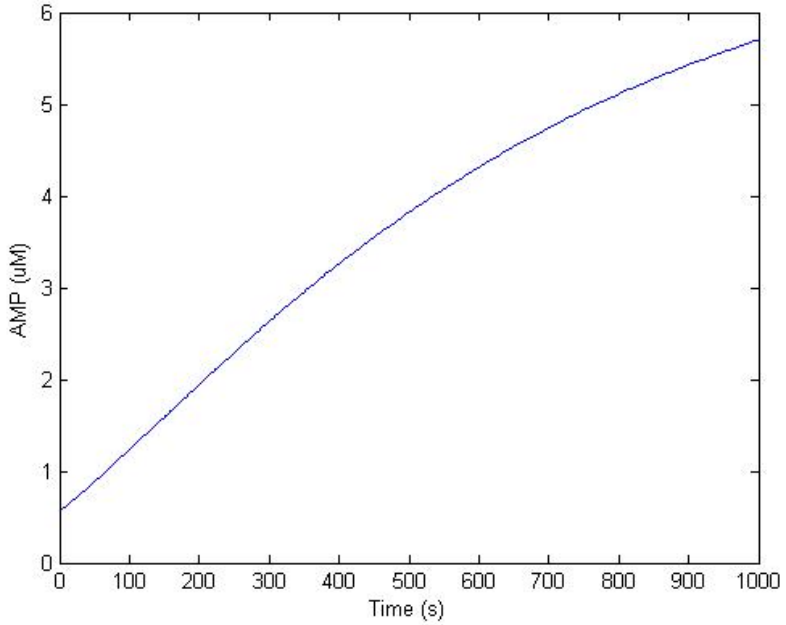
(We assume the system does not change ADP→ATP parameter to compensate for the effect of sAC inhibitor)

Bicarbonate-induced response of system pre-perfused with inhibitor of sAC

Virtual Experiment:

The system is allowed to equilibrate with a 50% reduction in sAC V_{max} , after which the near-maximal bicarbonate stimulus is applied.





Response to a bicarbonate-induced four-fold increase in basal inhibited sAC V_{max}

Summary…

Our hypothesis is plausible

-The system is working in the right regime of [AMP]

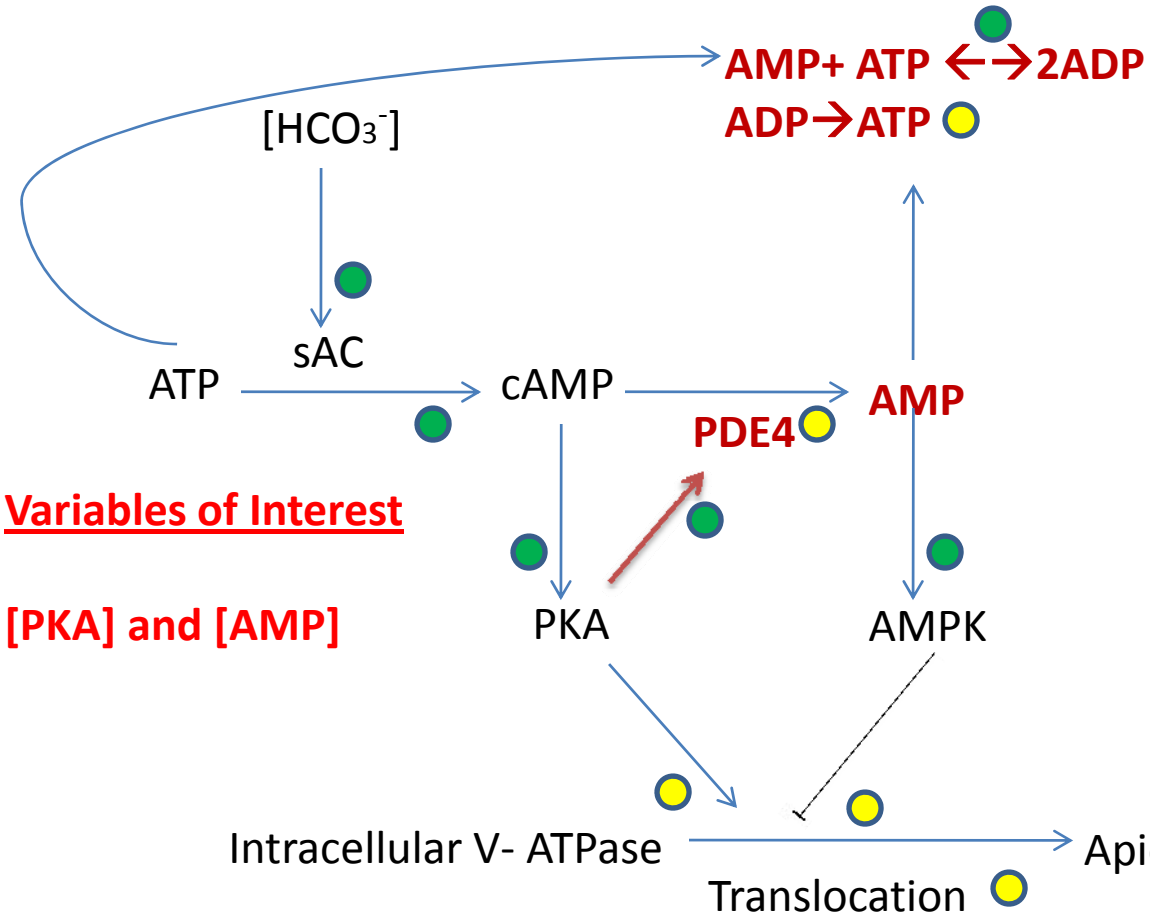
-With increased pH, the model predicts an increase in time window between start and stop of V-ATPase translocation.

Our conclusion is robust to the choice of PDE isoform is the model

We have used the model to suggest a wet-lab experiment to test the model boundary and estimate contributions of other mechanisms towards stopping the V-ATPase translocation

The AMP response is sensitive to the Vmax of sAC, but not to Km of PDE

Explanation of the Modeling Results



Stimulus
$[HCO_3^-]$ ↑
pH ↑

Normal
pH=6.5
Low $[HCO_3^-]$

The scheme contains assumed chemical species and reactions (maroon).
Green dot: parameters are known for the reaction.
Yellow dot: Parameters are unknown for the reaction.

Implications of the Study (Once Completed)

-For the first time, the study may connect the canonical Adenylyl cyclase-cAMP pathway from the cAMP signaling field and the canonical AMP pathway from the metabolic field

-If successful, the study would provide insights into the regulation of V-ATPase mediated pH homeostasis, and associated diseased states such as alkalosis and male infertility.