Tight regulation of electrically-charged substrate transport in bacteria

Emory University

Minsu Kim

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I will talk about one problem related to electric charge when a cell strives to achieve its dream.

What does it take for one cell to become two cells?

Biomass of a bacterial cell: ~50% Carbon, ~15% Nitrogen . .

Carbon and nitrogen uptake

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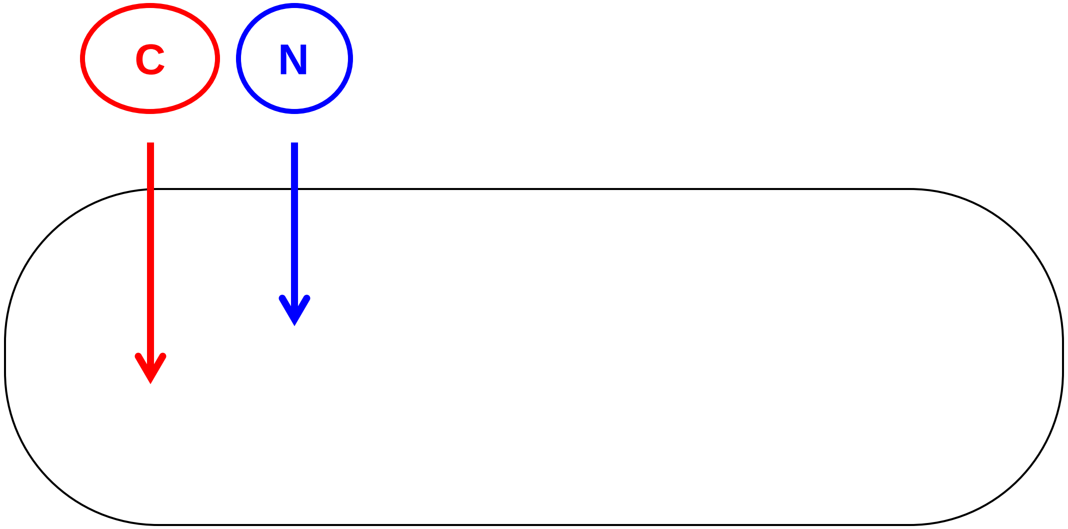

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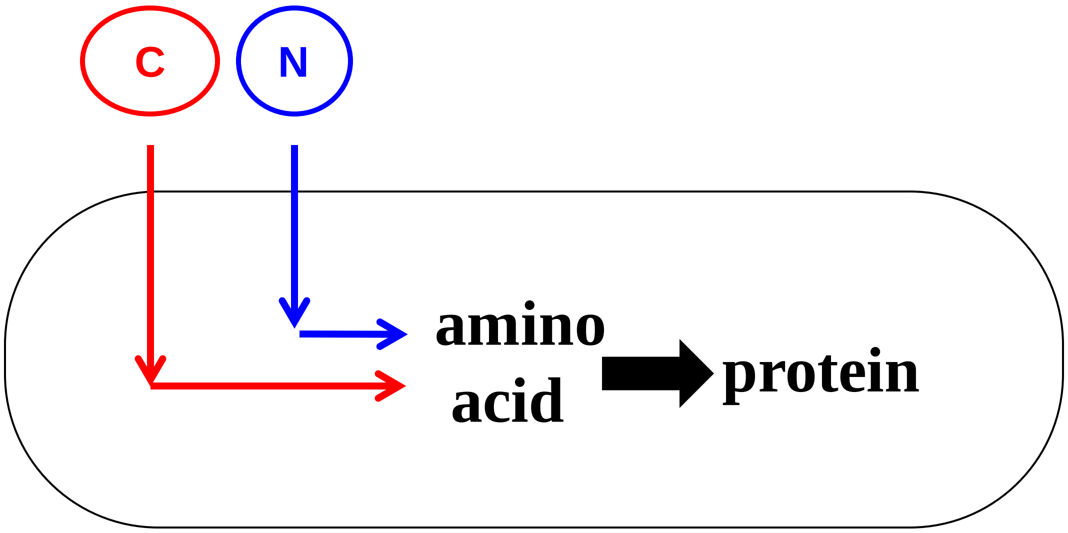

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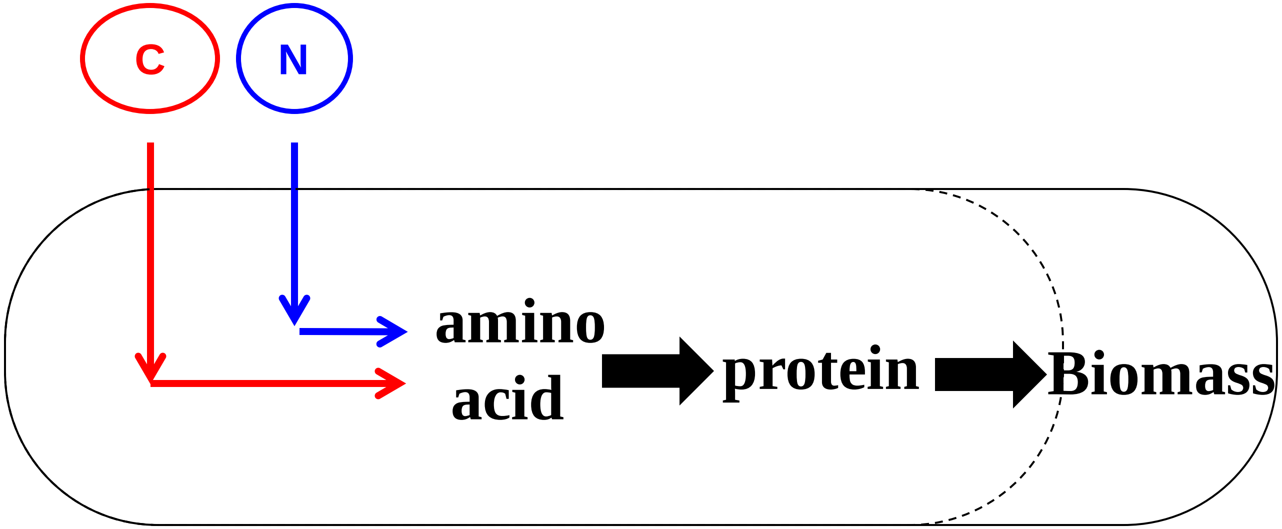

Carbon and nitrogen uptake is important

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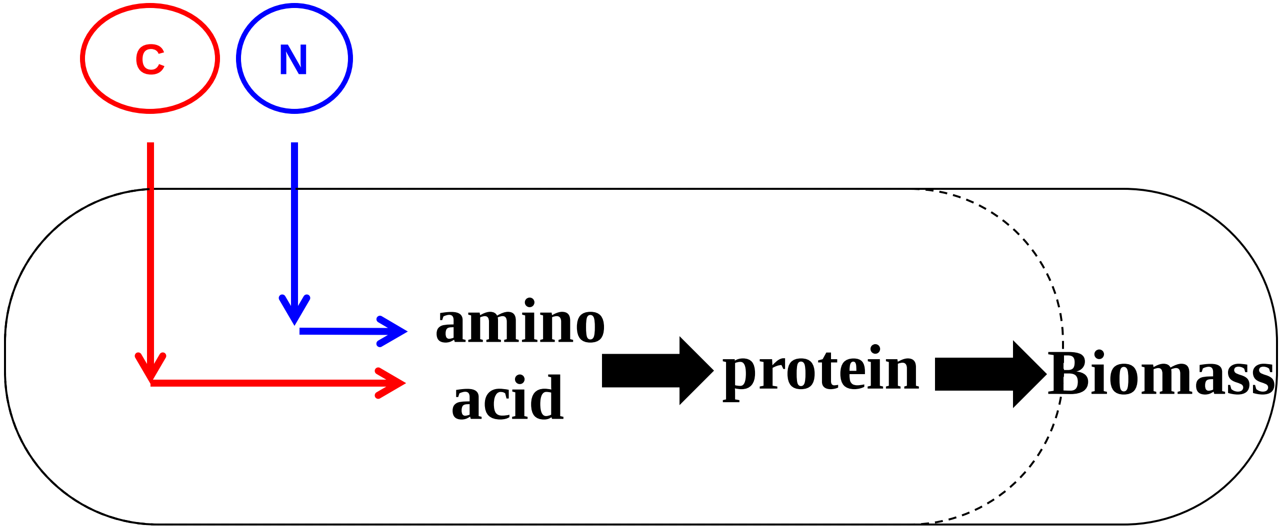

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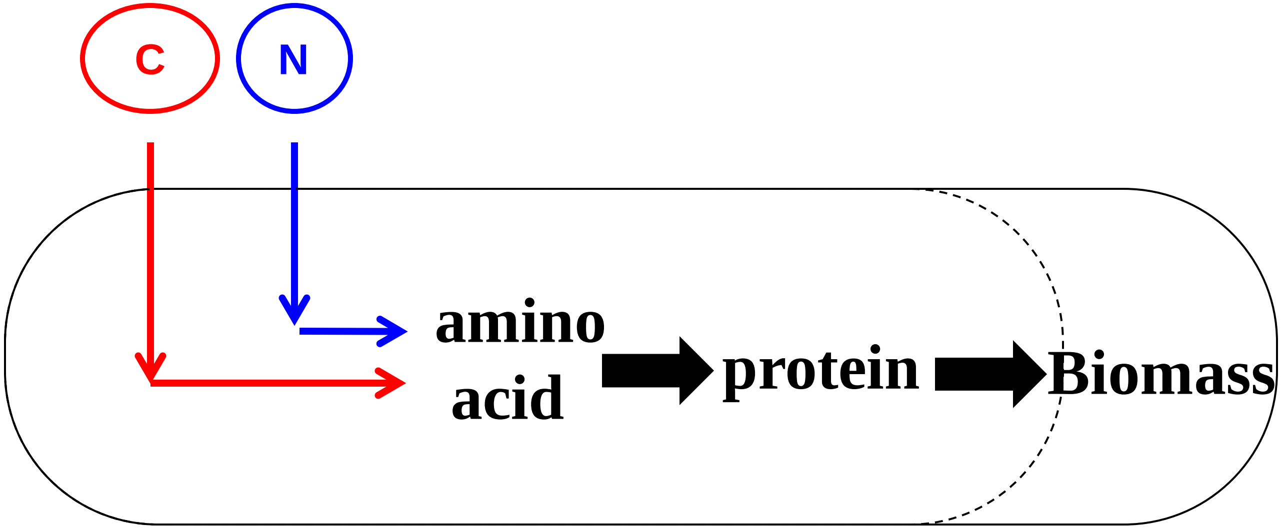

Carbon uptake and metabolism have been extensively characterized.

However, much less is known about nitrogen uptake

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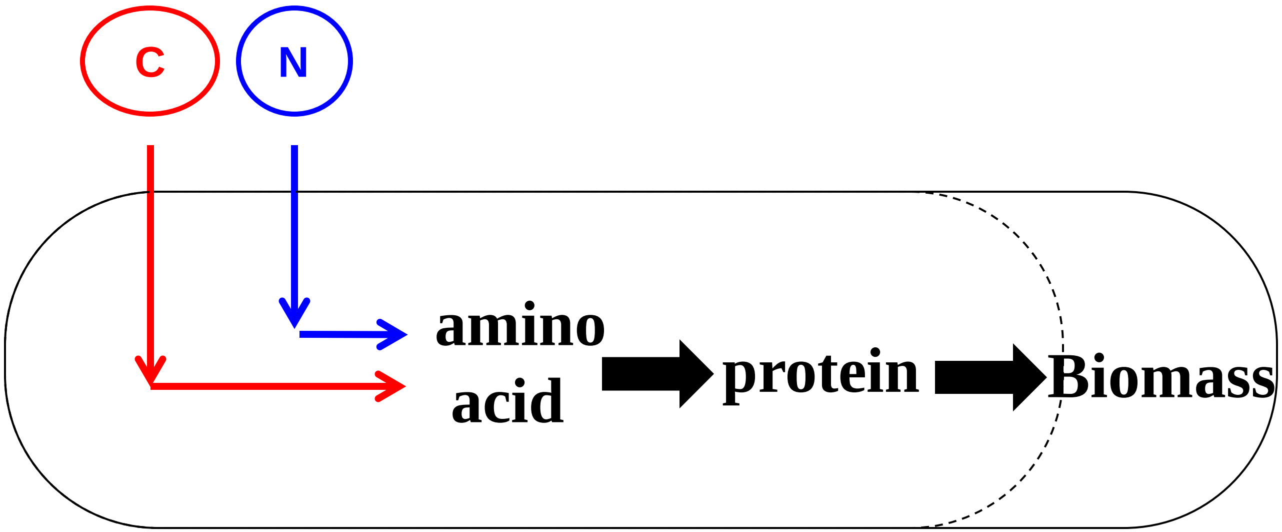

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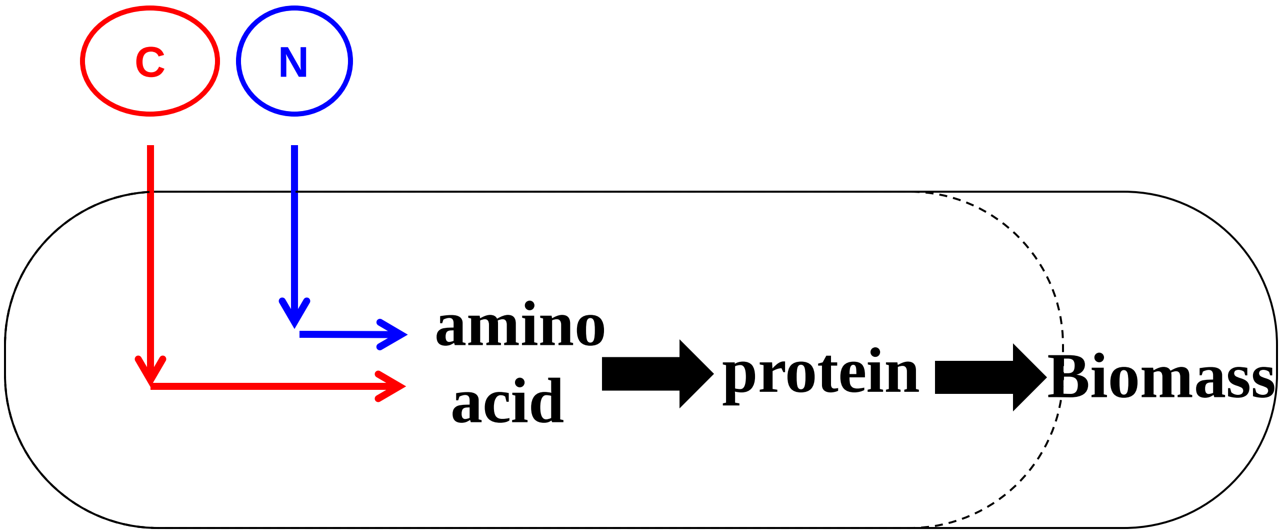

Nitrogen uptake is peculiar.

Nitrogen metabolism and regulation

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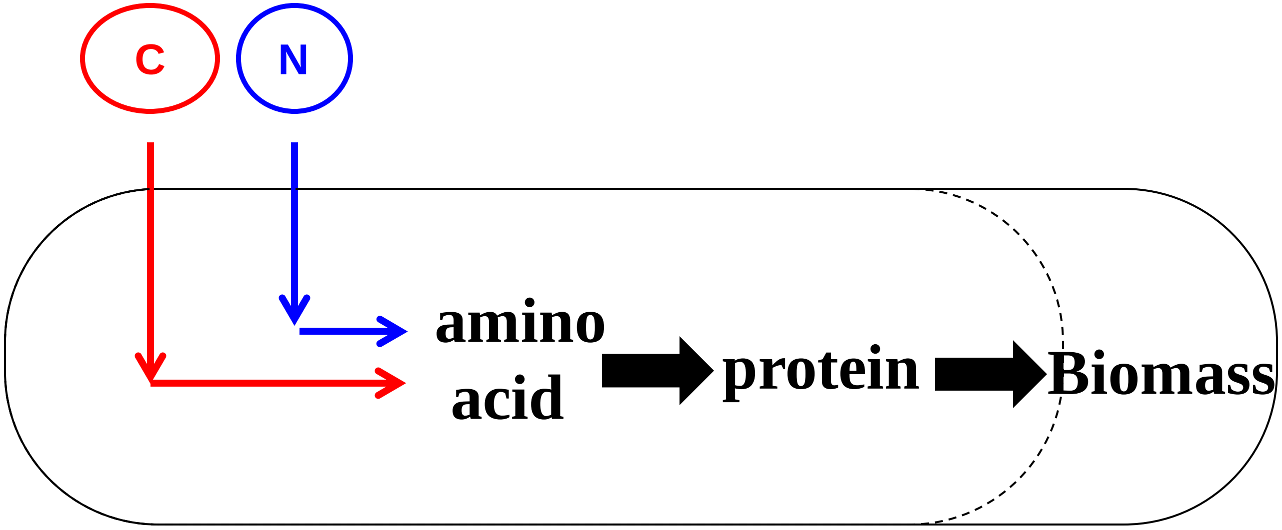
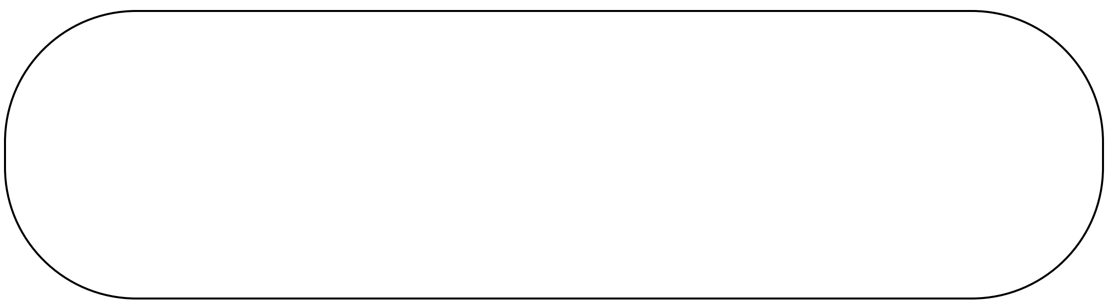


NH_4^+/NH_3 $NH_4^+ \leftrightarrow H^+ + NH_3$, $[NH_4^+]/[NH_3]$~180 at pH 7

NH_3 passive diffusion can limit growth

NH_3 is very permeable to membrane (~30 times more than H_2O)

$$NH_3 \longleftrightarrow NH_4^+$$



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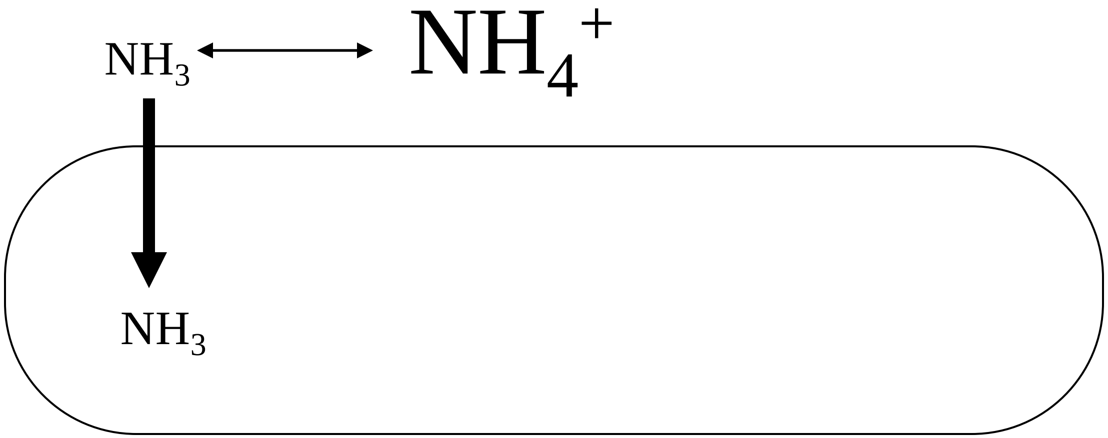

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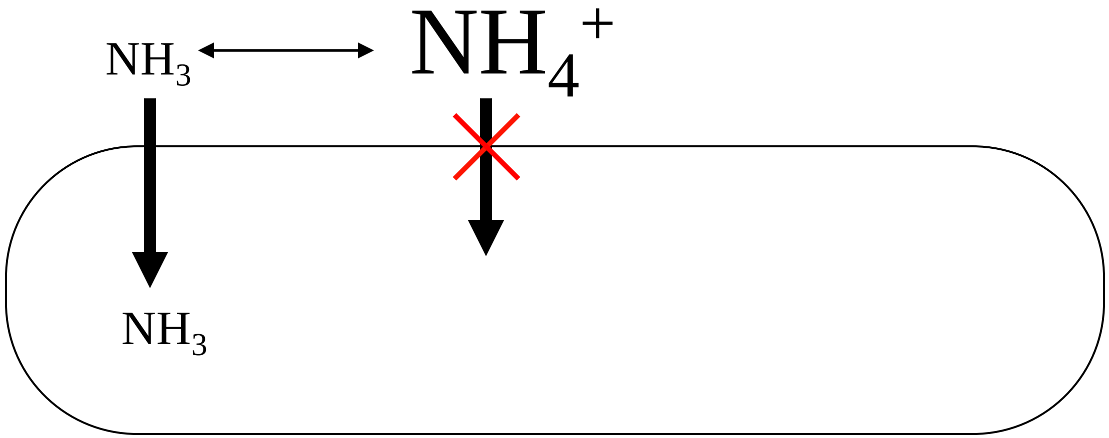

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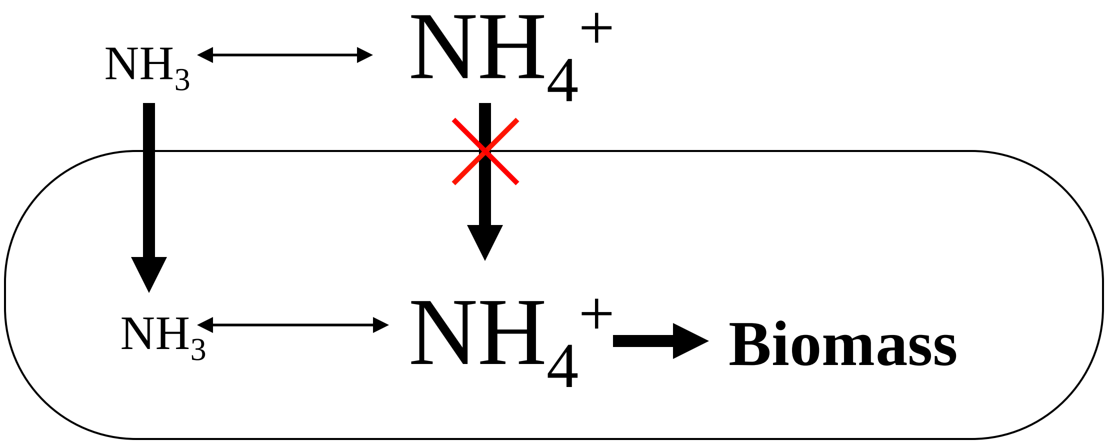

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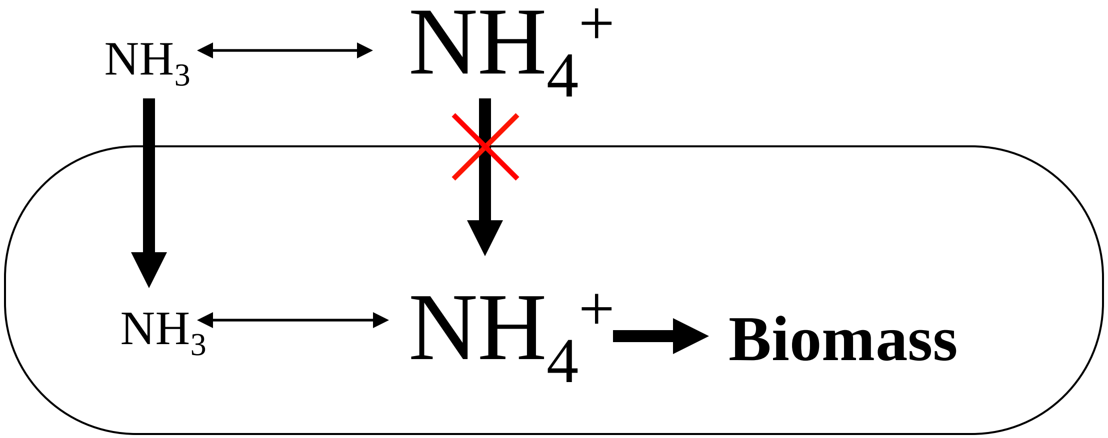

NH_3 passive diffusion can limit growth

AmtB transports NH_4^+

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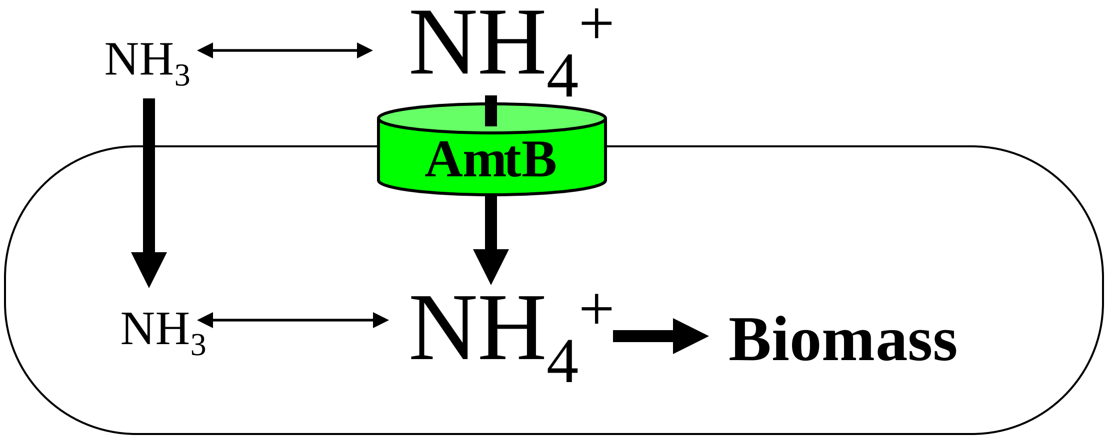
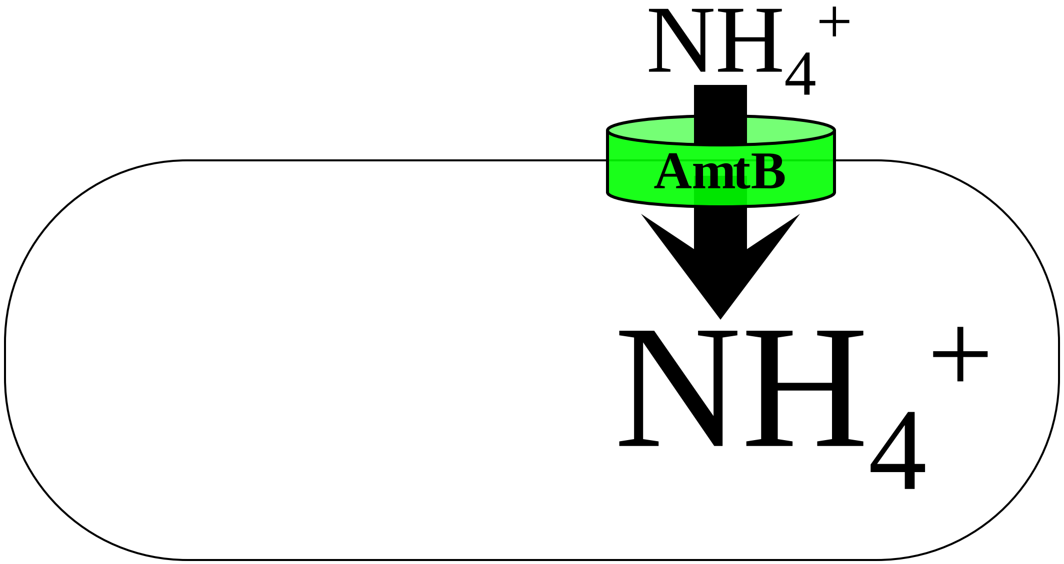


AmtB transports and concentrates NH_4^+ internally

Concentrate?

NH_3 is very permeable to membrane.



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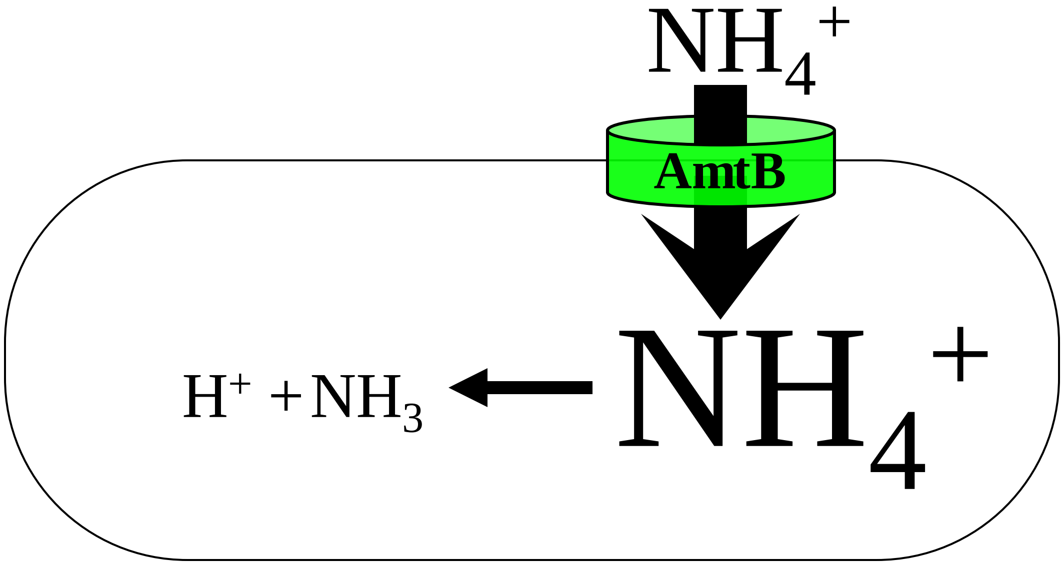

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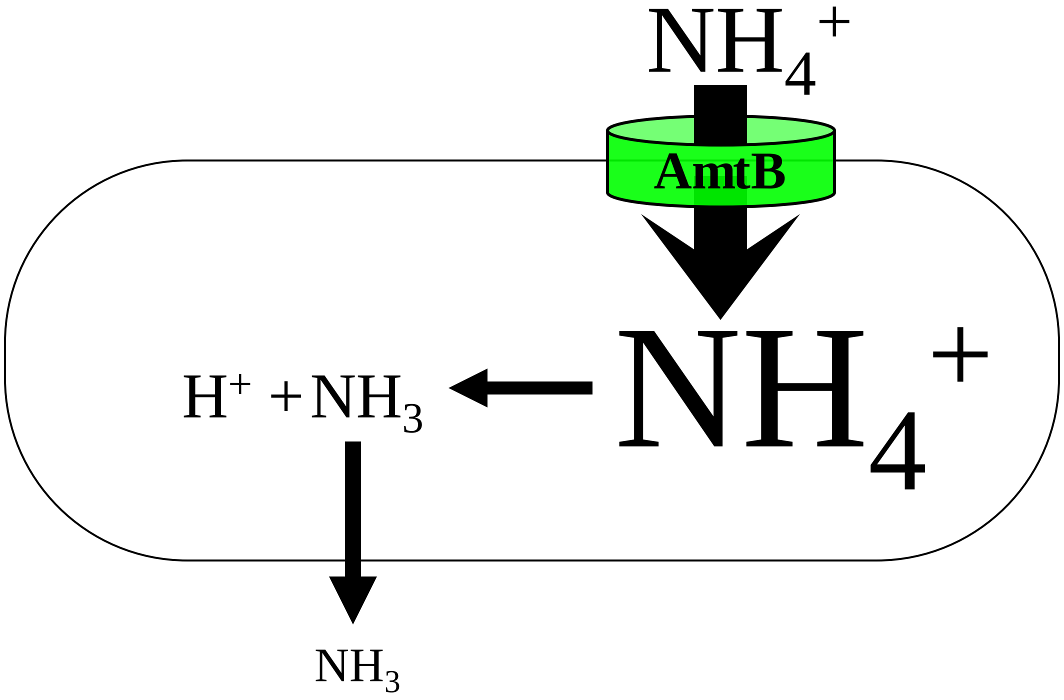

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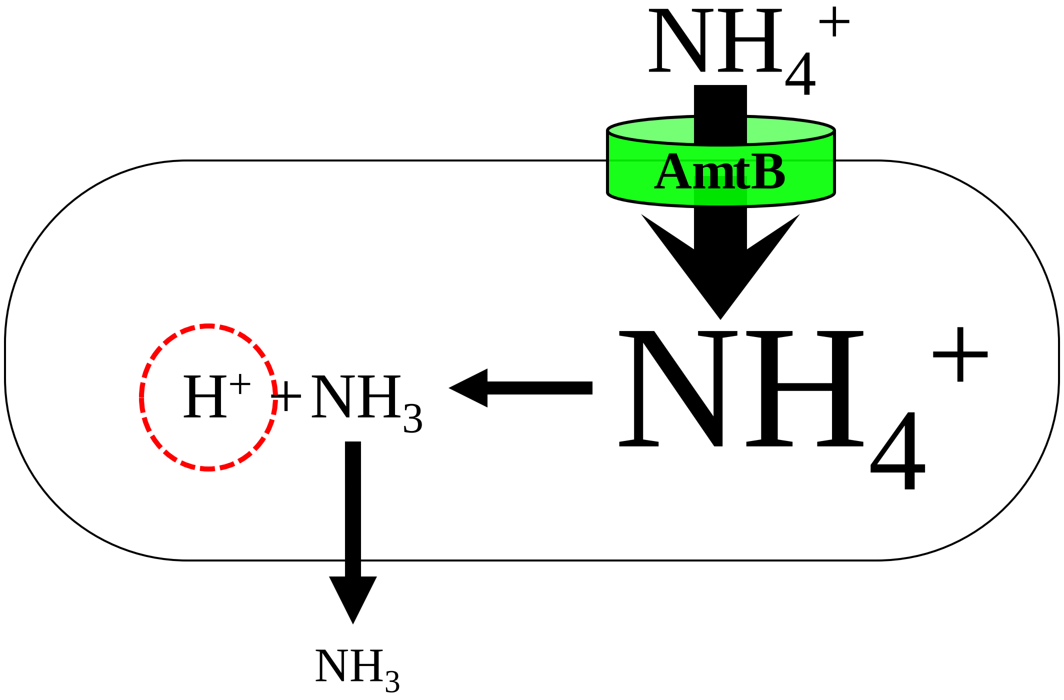

AmtB may be harmful

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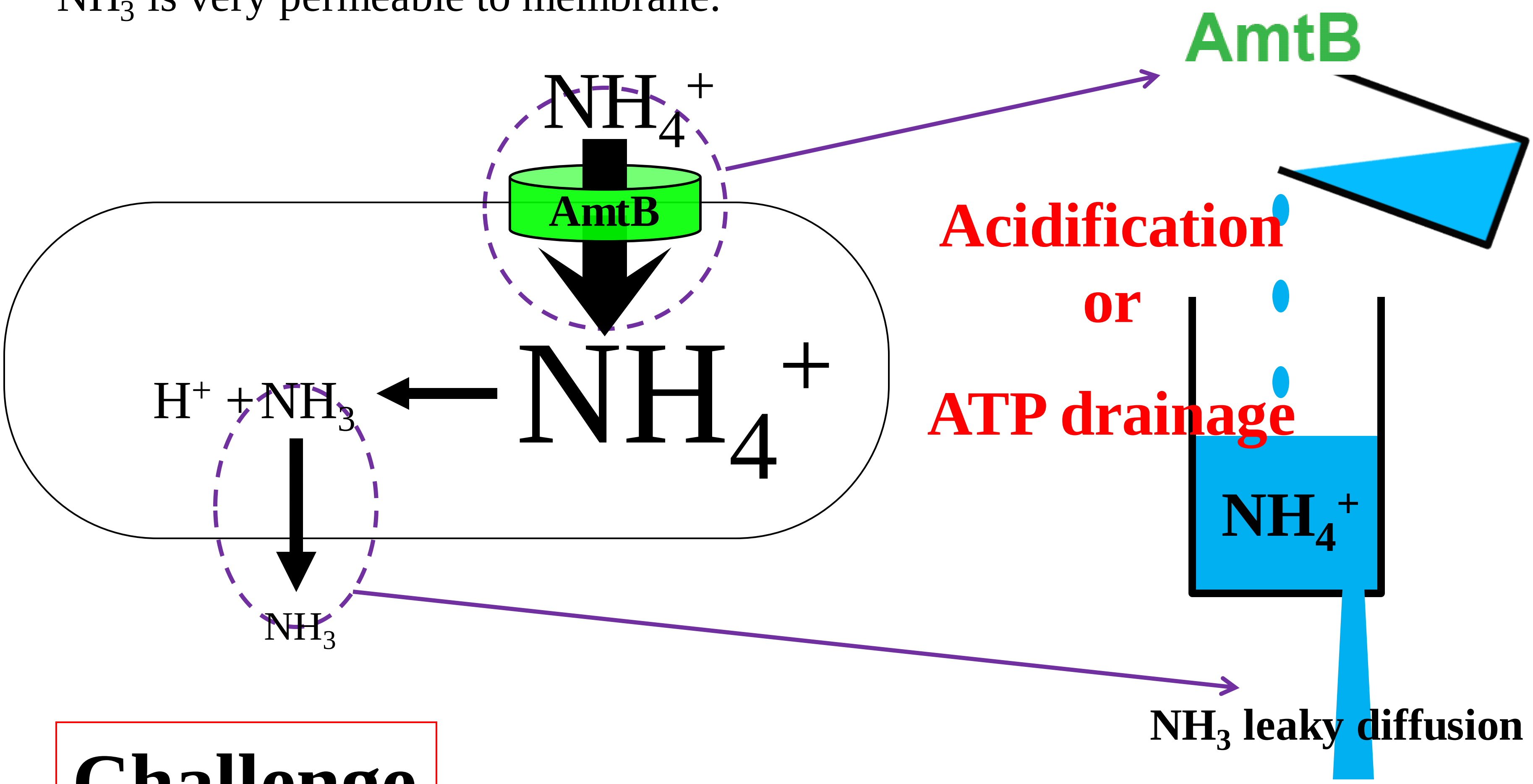

Challenge

AmtB may be harmful

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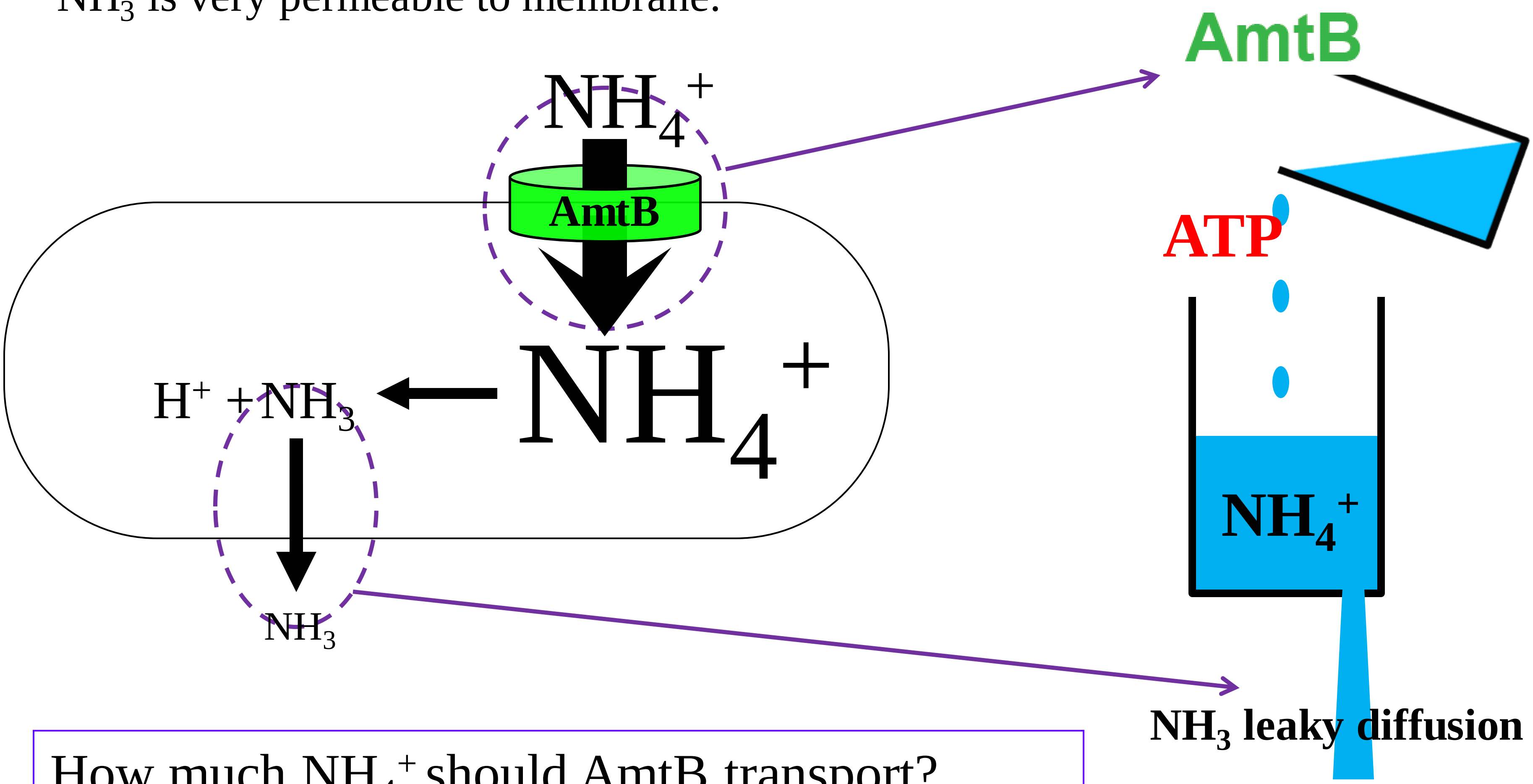
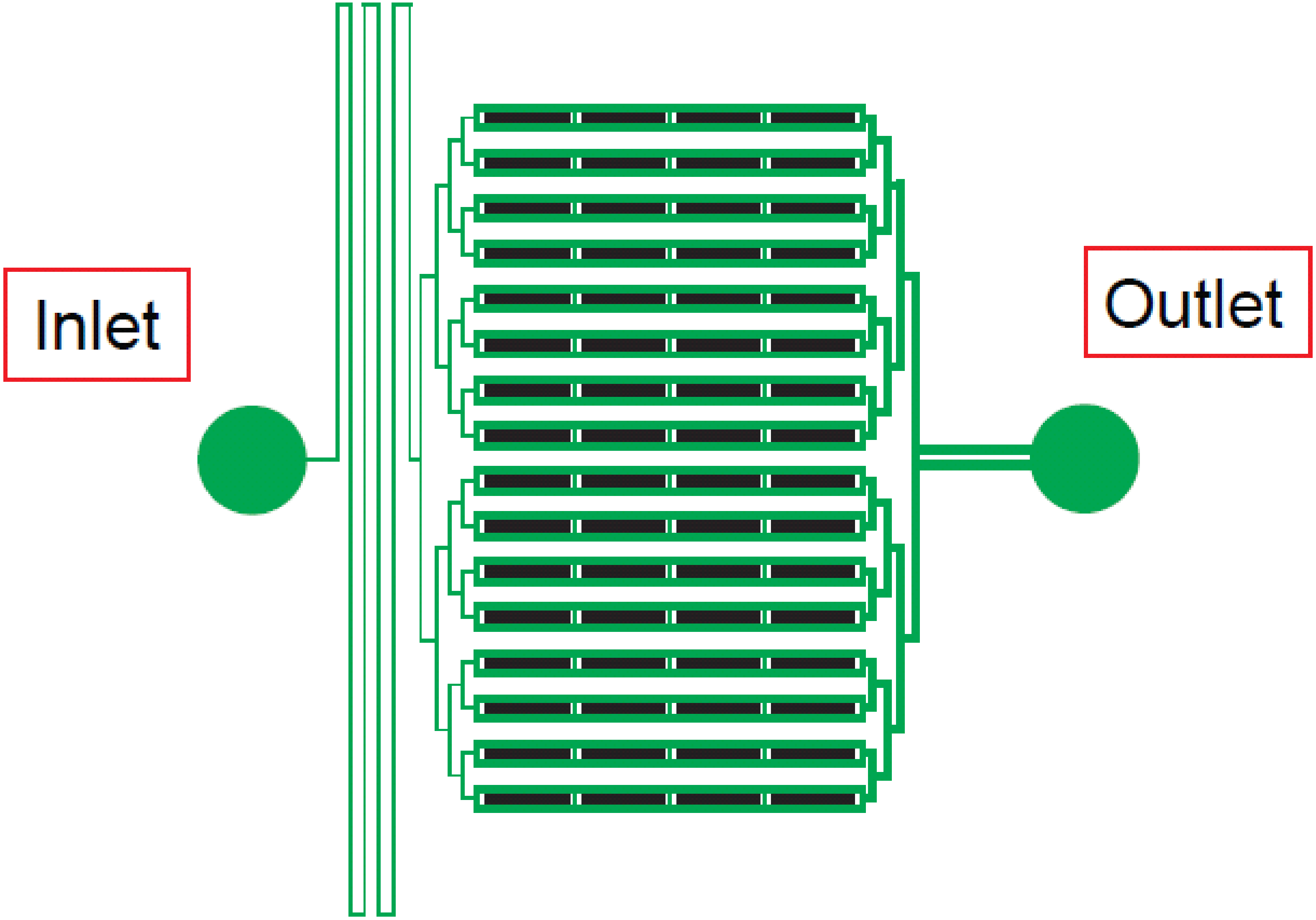


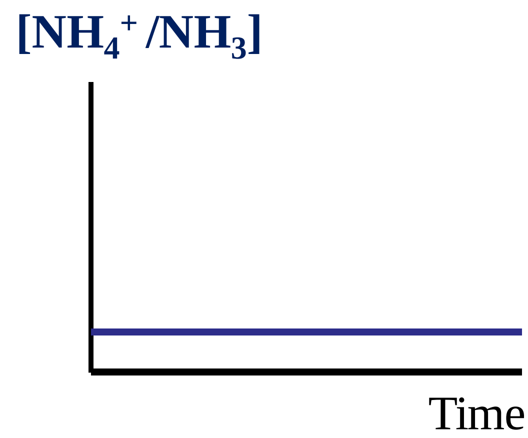
How much NH_4^+ should AmtB transport?
How does *E. coli* regulate it?

Microfluidic chemostat

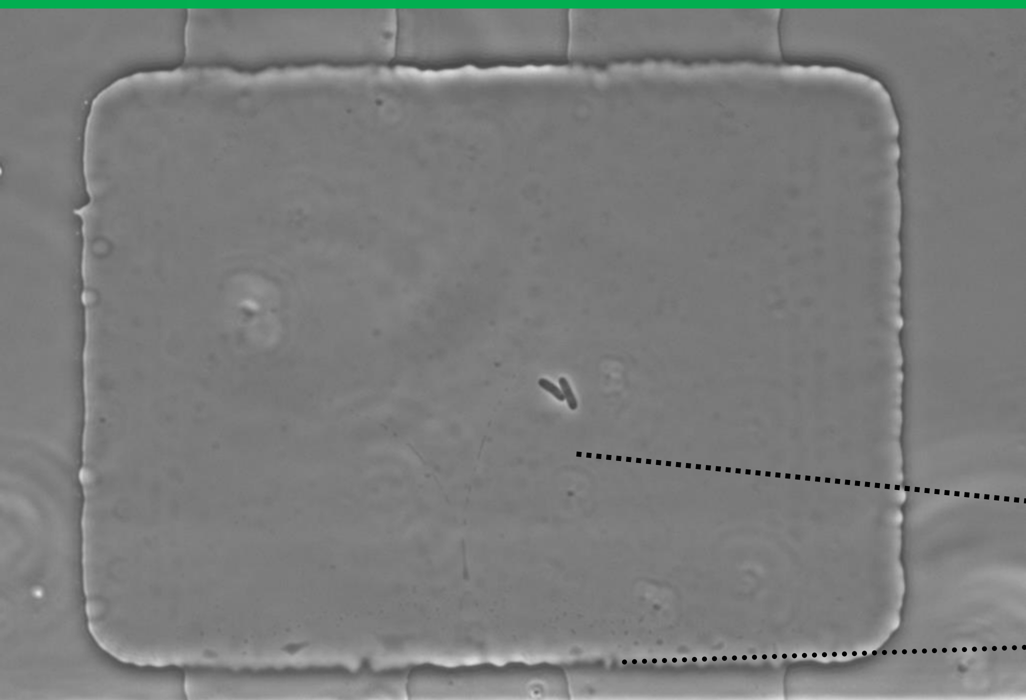


Microfluidic chamber

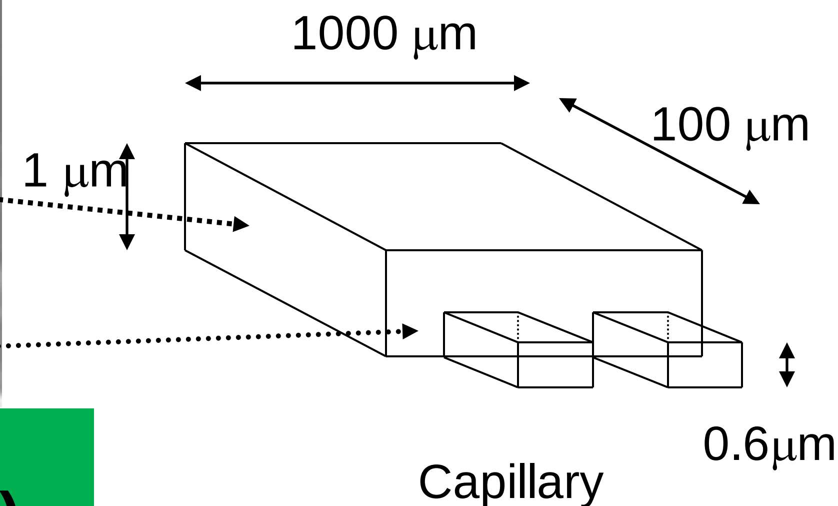
E. coli NCM3722, in Rich Defined Medium +0.5% glycerol



Channel (Flow of fresh medium)

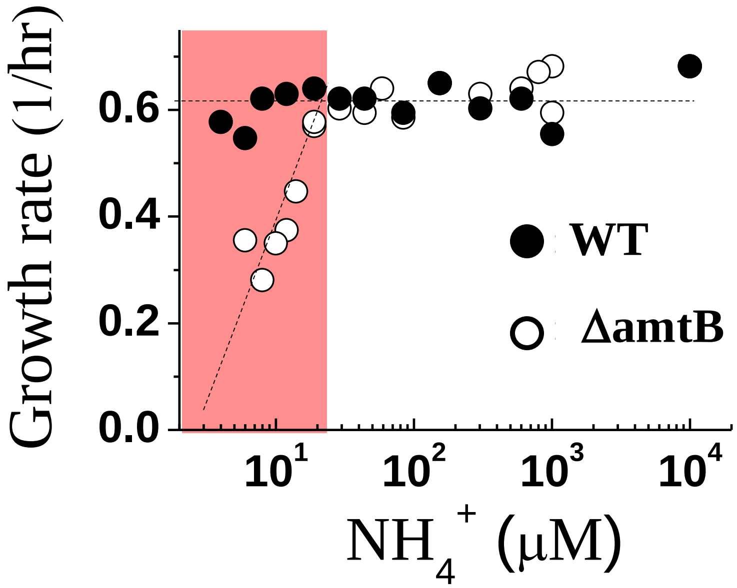


Nutrient exchange rate: ~40s



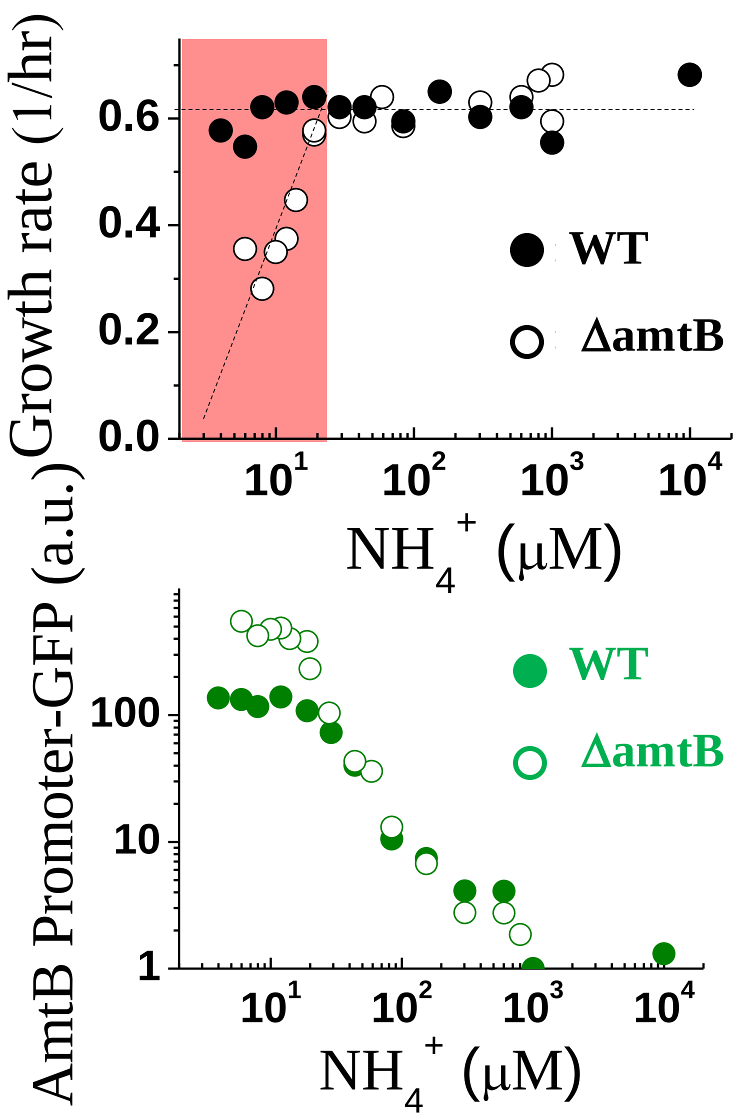
Channel (Flow of fresh medium)

Growth rate with and without AmtB



E. coli K-12 NCM3722 in Neidhardt's MOPS medium with 0.4% of glyc with various ammonium concentrations.

AmtB promoter-GFP



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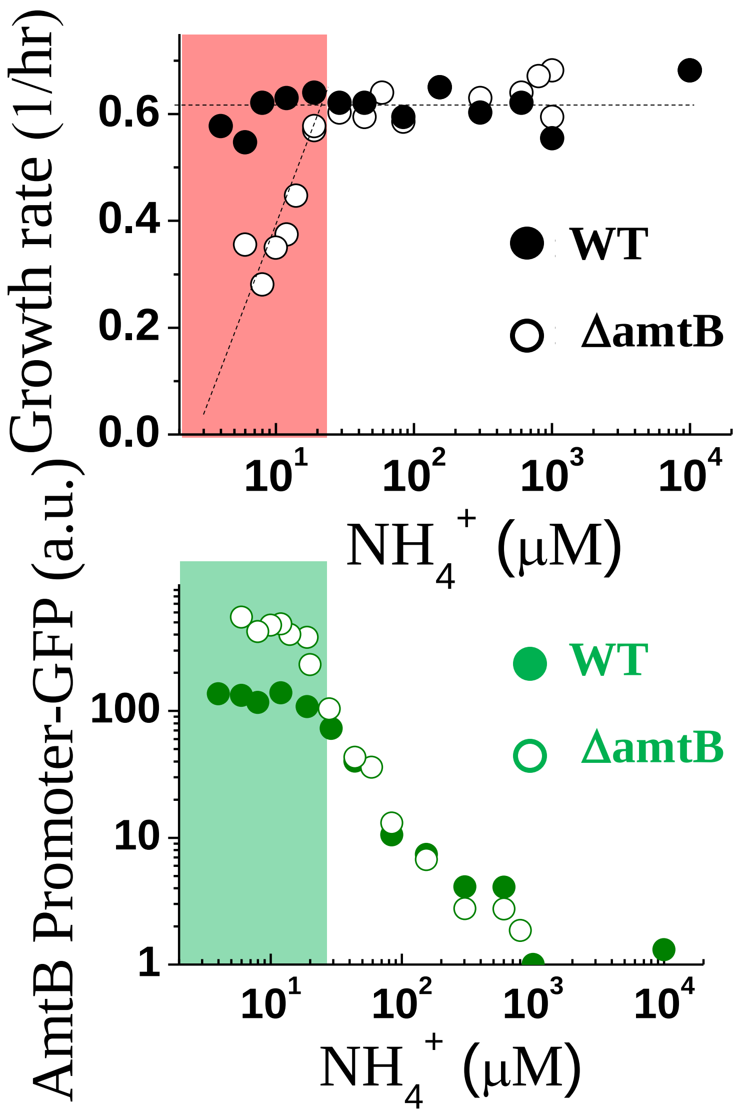

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AmtB promoter activities deviate for the two strains.

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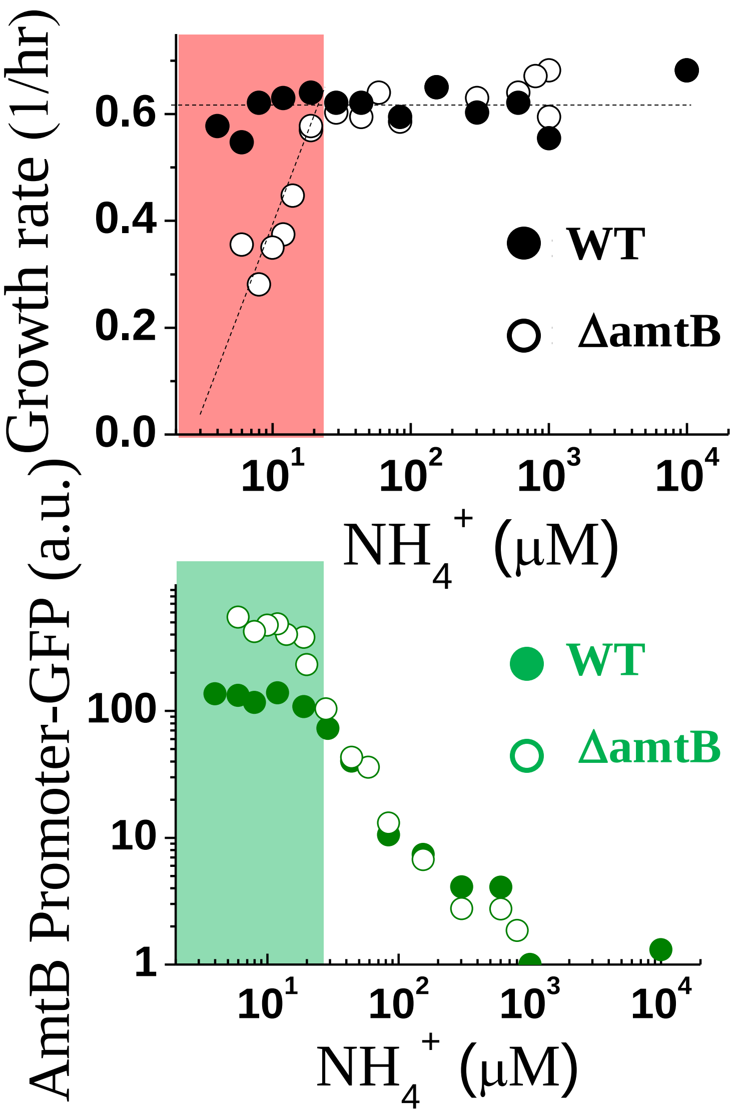

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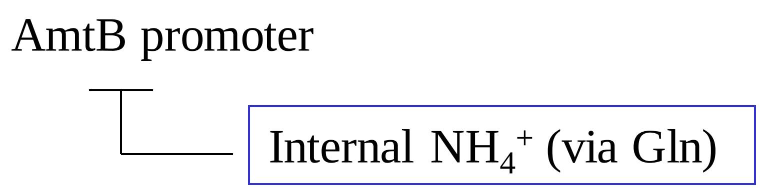
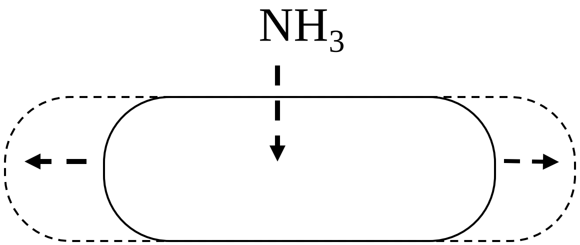


AmtB promoter activities deviate for the two strains.

Quantitative analysis

ΔamtB



N assimilation into biomass = NH_3 diffusion

N_0 = # of Nitrogen per cell
λ = Growth rate

$$N_0 \cdot \lambda \quad \Longleftrightarrow \quad P \cdot ([NH_3]^{ext} - [NH_3]^{int})$$

P: membrane permeability

$$N_0 \cdot \lambda_\Delta = C \cdot P \cdot ([NH_4^+]_\Delta^{ext} - [NH_4^+]_\Delta^{int}) \quad \cdots \ (1)$$

C: conversion factor

Quantitative analysis

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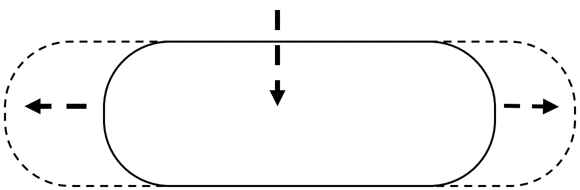

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WT

N assimilation into biomass = NH_3 diffusion + NH_4^+ transport

$$N_0 \cdot \lambda_{wt} = C \cdot P \cdot ([NH_4^+]_{wt}^{ext} - [NH_4^+]_{wt}^{int}) + J_{AmtB} \quad \cdots \ (2)$$

Quantitative analysis

ΔamtB

$$N_0 \cdot \lambda_{\Delta} = C \cdot P \cdot ([\mathrm{NH}_4^+]_{\Delta}^{\mathrm{ext}} - [\mathrm{NH}_4^+]_{\Delta}^{\mathrm{int}}) \quad \cdots \ (1)$$

WT

P=0.13cm/sec
Walter and Gutknecht(1986)

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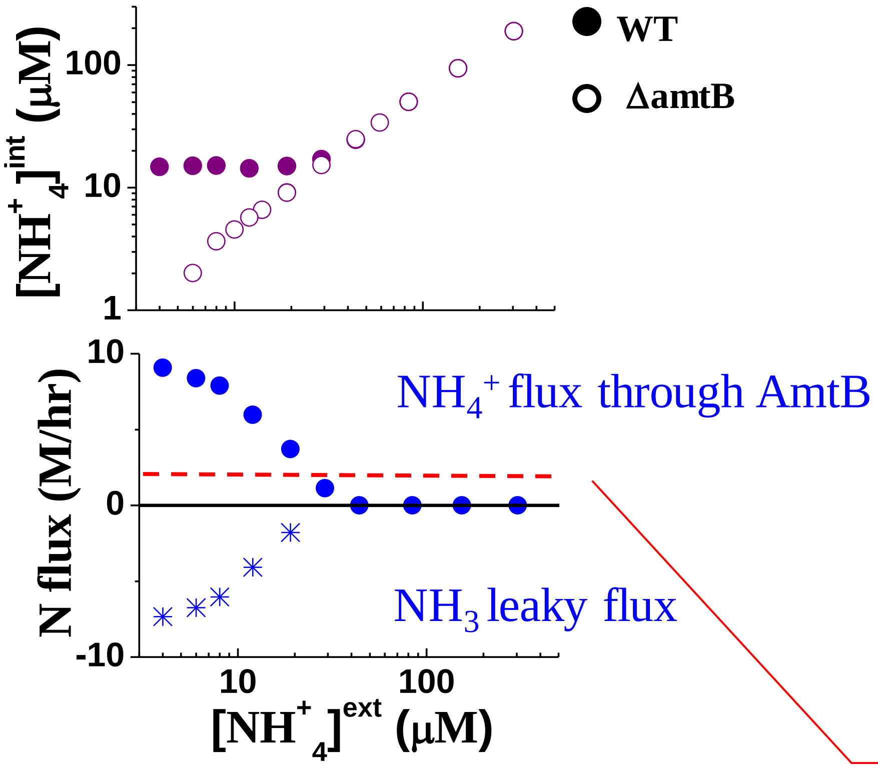
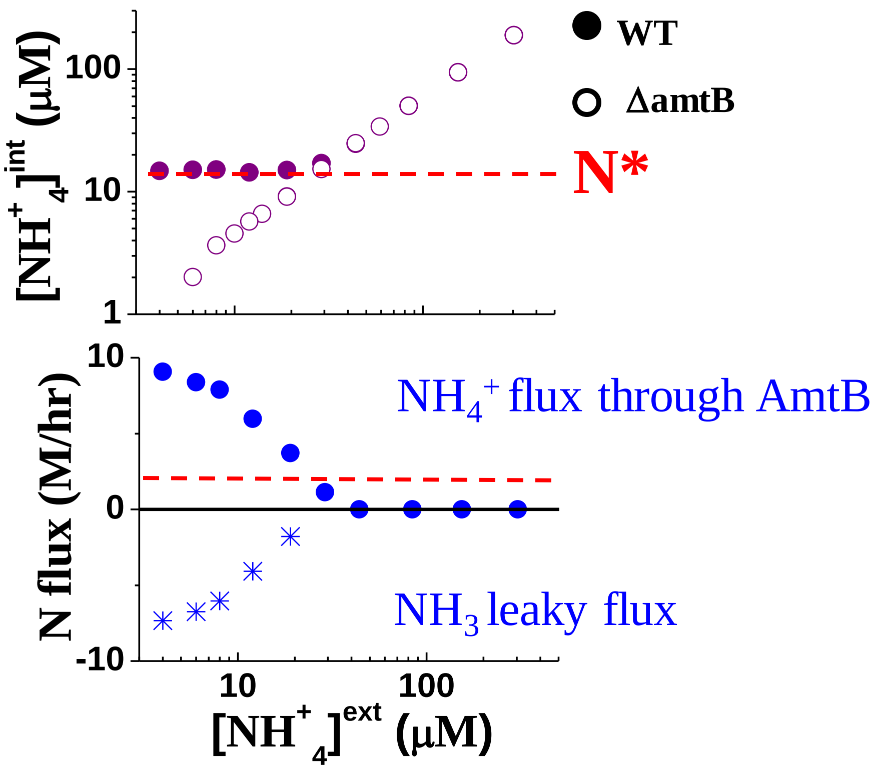
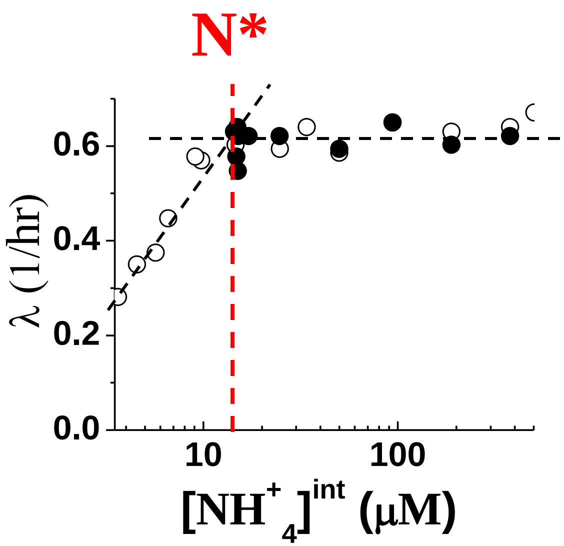
$[NH_4^+]^{int}$ and J_{AmtB}



For 65min DT growth

$[NH_4^+]^{int}$ and J_{AmtB}





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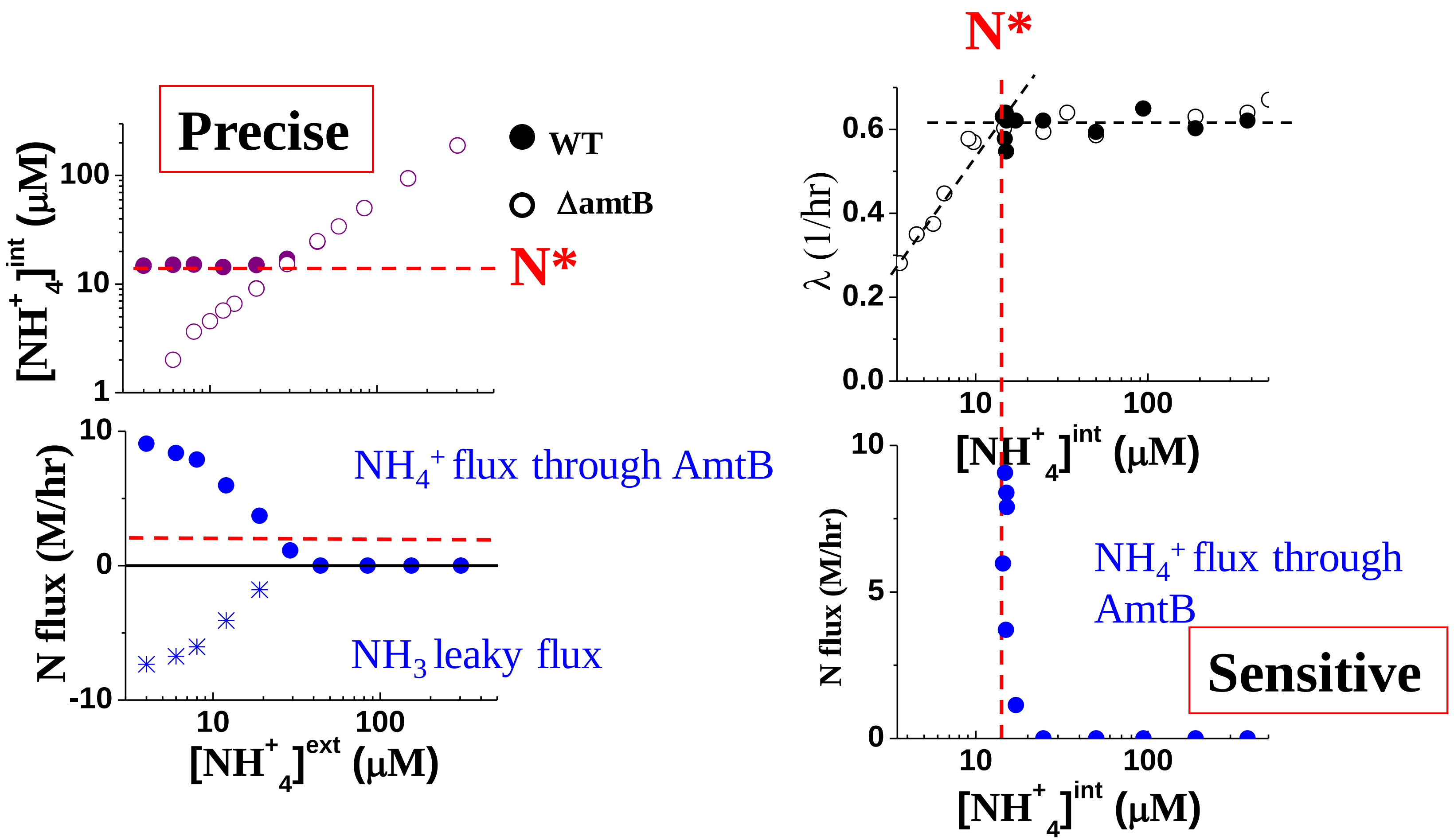
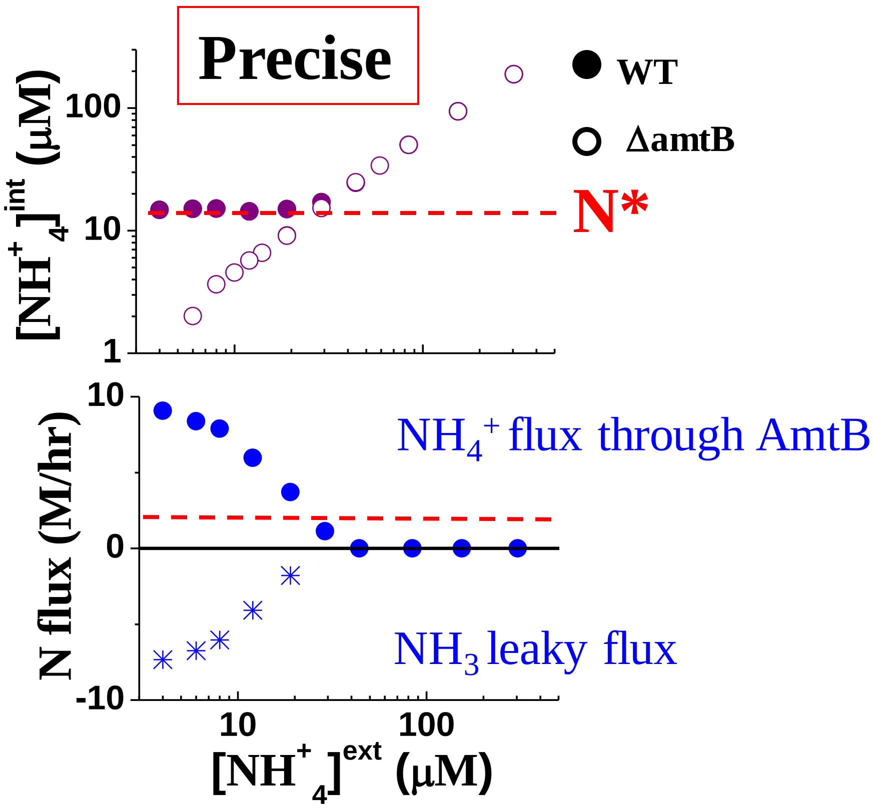
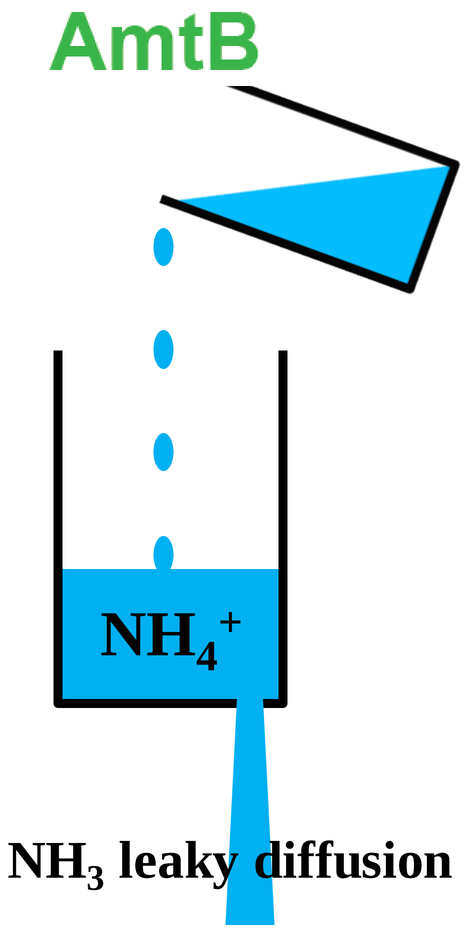


AmtB is regulated very <u>sensitively</u> so that internal ammonium is maintained <u>precisely</u> at the minimal level needed for the optimal growth.

$[NH_4^+]^{int}$ and J_{AmtB}





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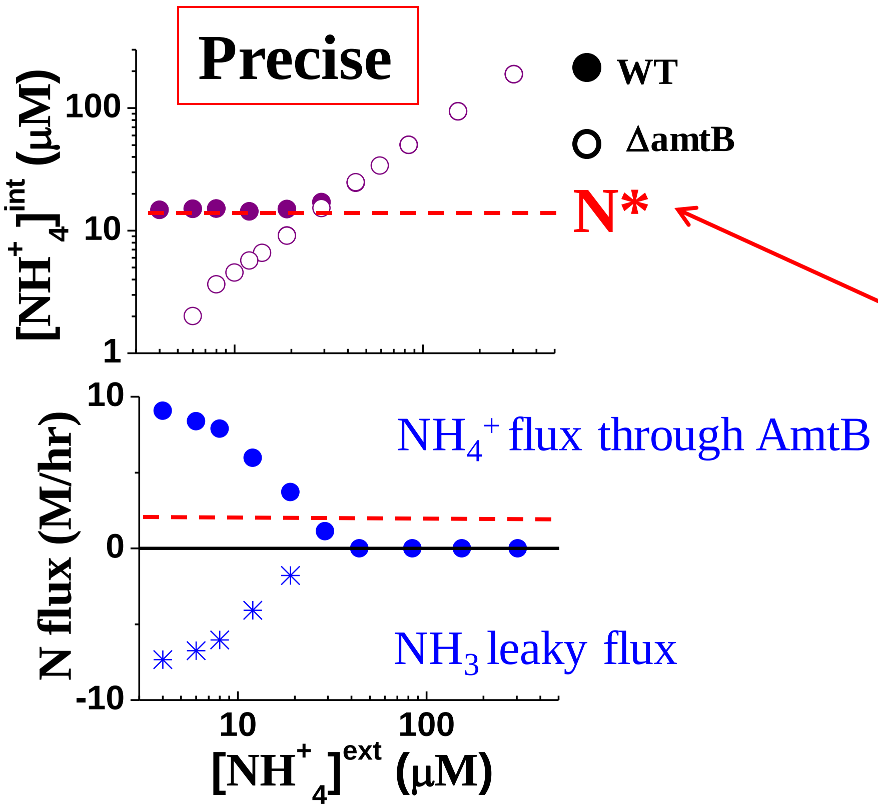

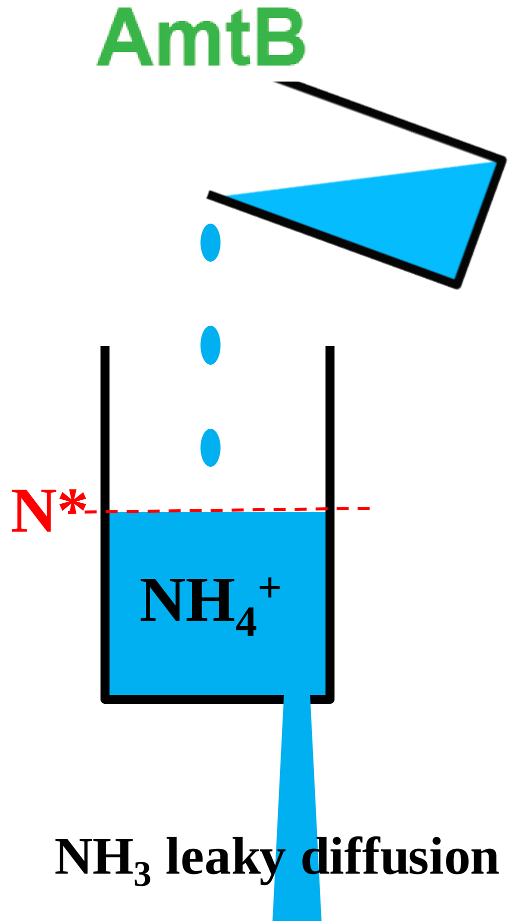

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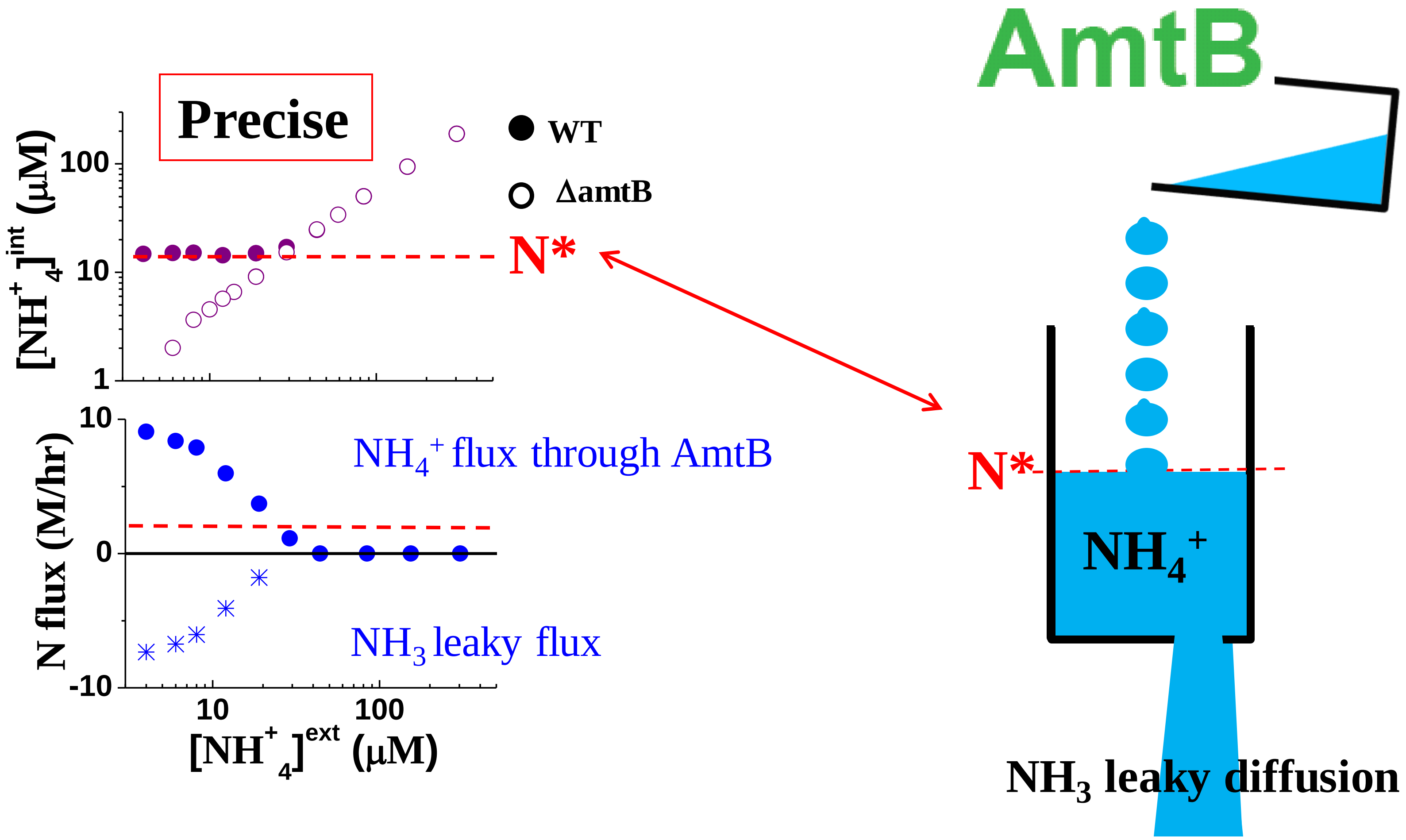
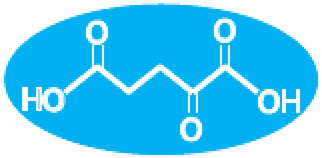
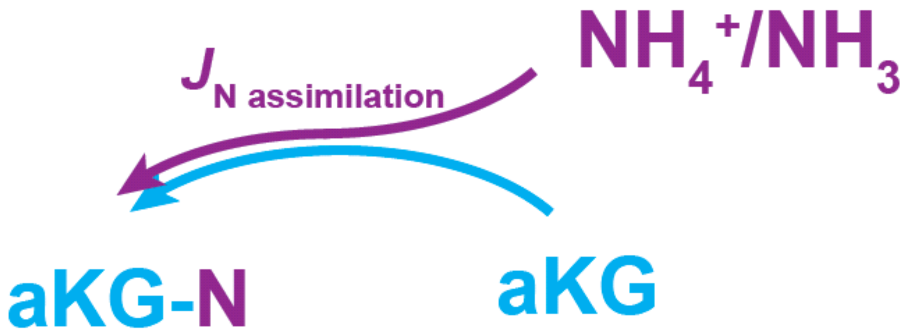


Putting together known interactions

aKG : alpha-ketoglutarate.



aKG: a nitrogen carrier



$$\frac{d}{dt}[\mathrm{aKG}] = J_{\mathrm{biomass}} - J_{\mathrm{N\ assimilation}} \longrightarrow [\mathrm{aKG}] = \int J_{\mathrm{biomass}} - J_{\mathrm{N\ assimilation}}\ dt$$

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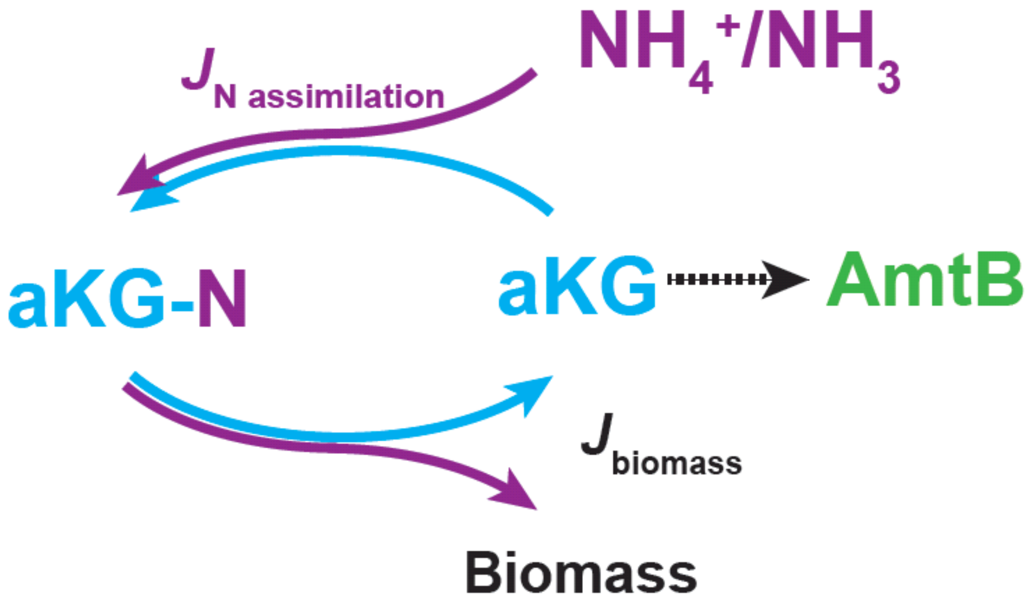

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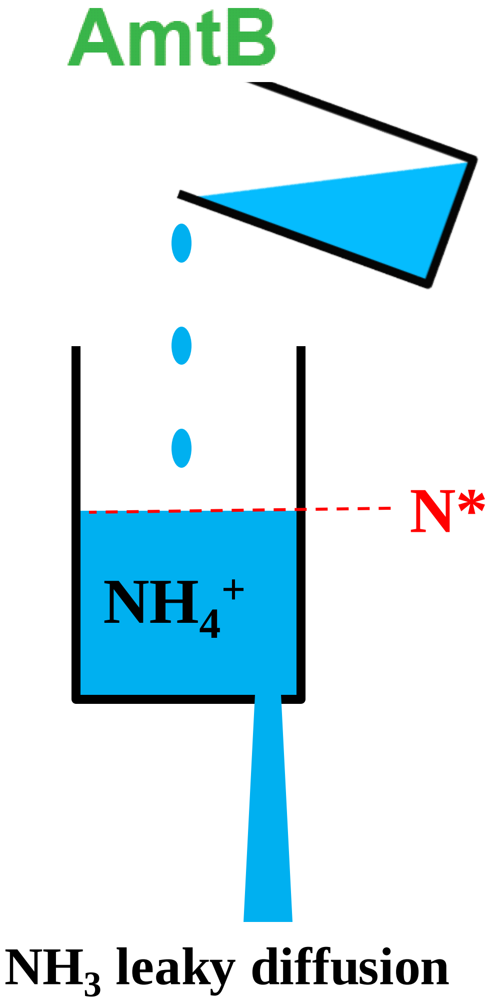
External ammonium ↓ ⇒ Leaky diffusion↑



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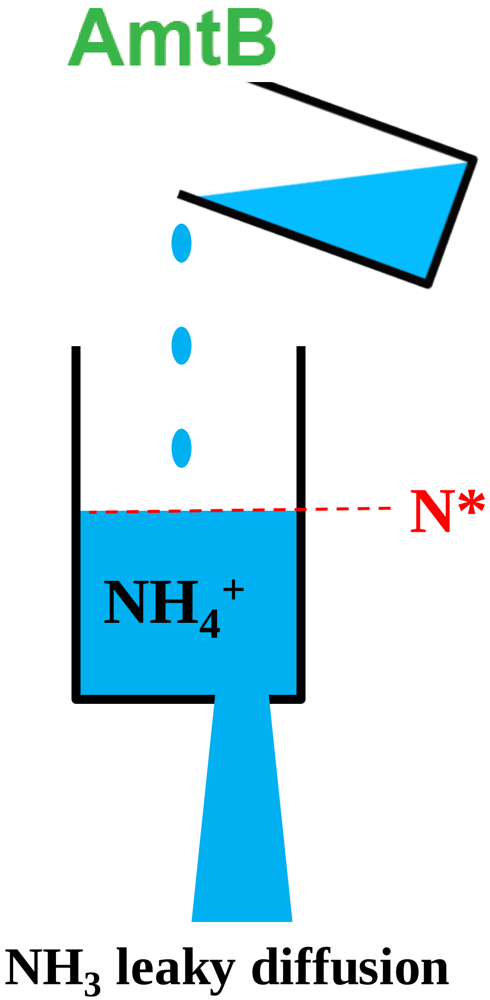
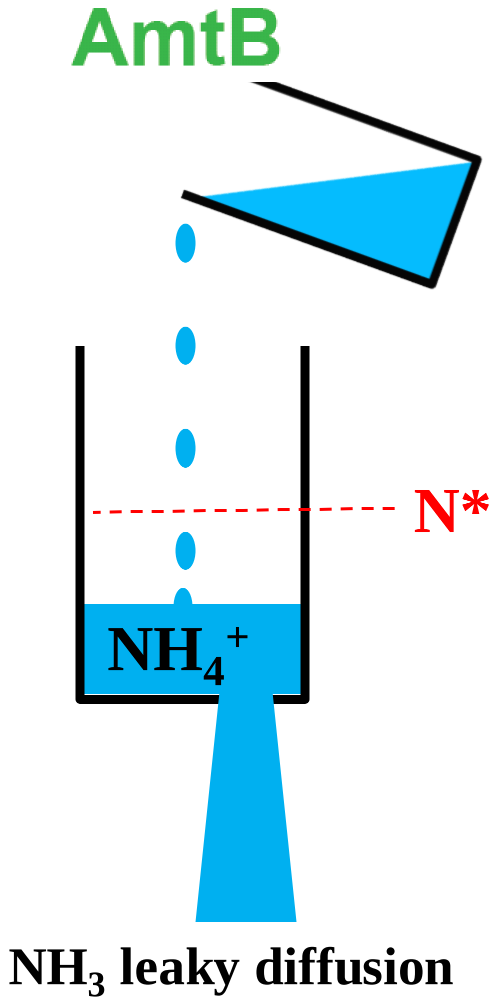
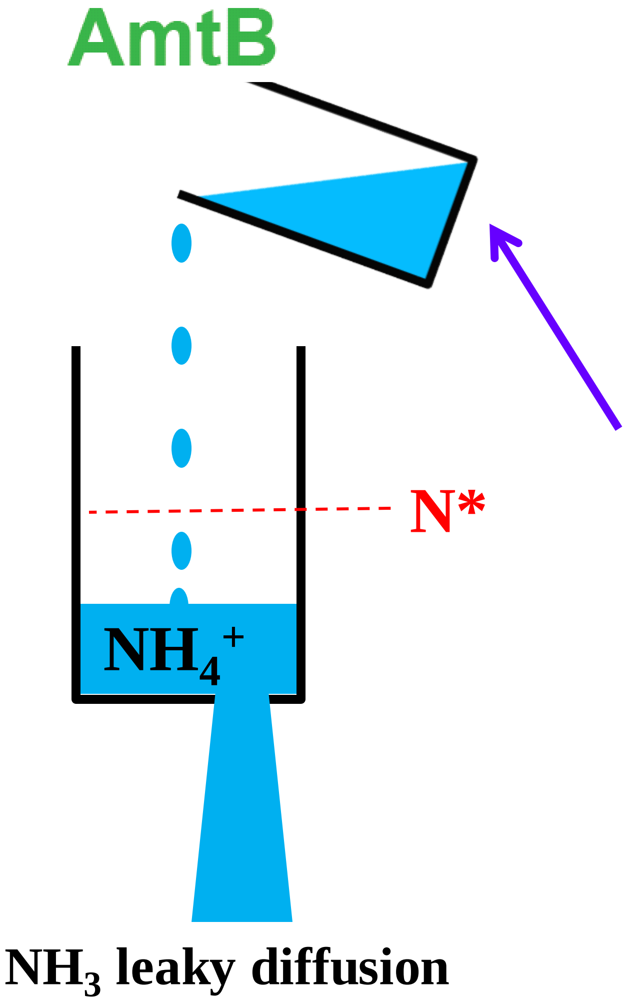


Leaky diffusion↑ ⇒ $[NH_4^+]^{int}$ ↓

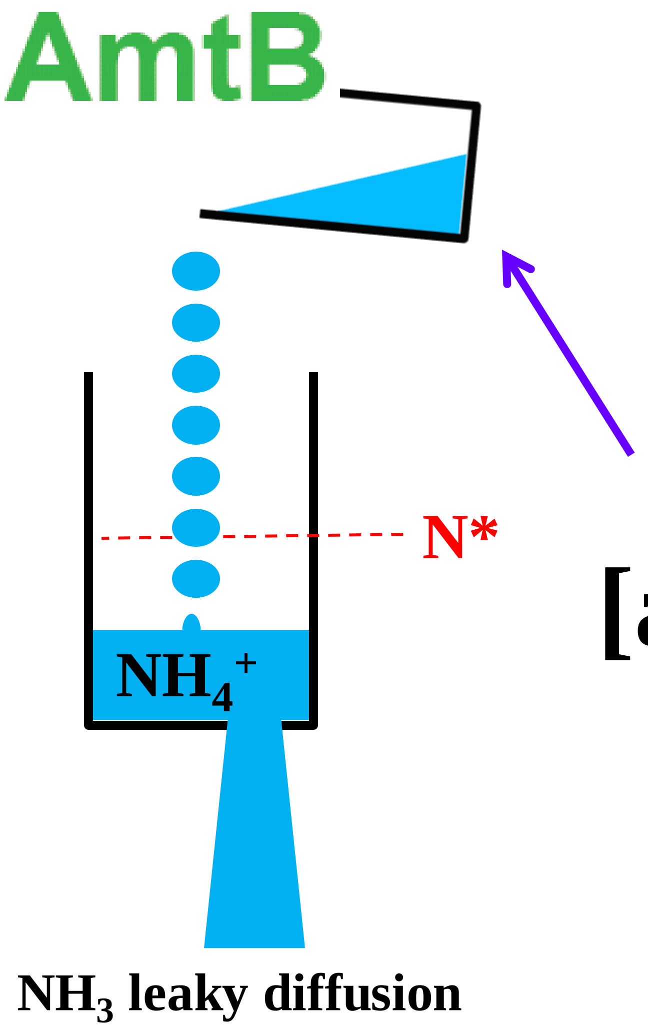


$[aKG]\uparrow \Rightarrow AmtB\uparrow$



$$[aKG] = \int J_{biomass} - J_{N\ assimilation}\ dt$$

$\text{AmtB}\uparrow \Rightarrow [NH_4^+]^{int}\uparrow$



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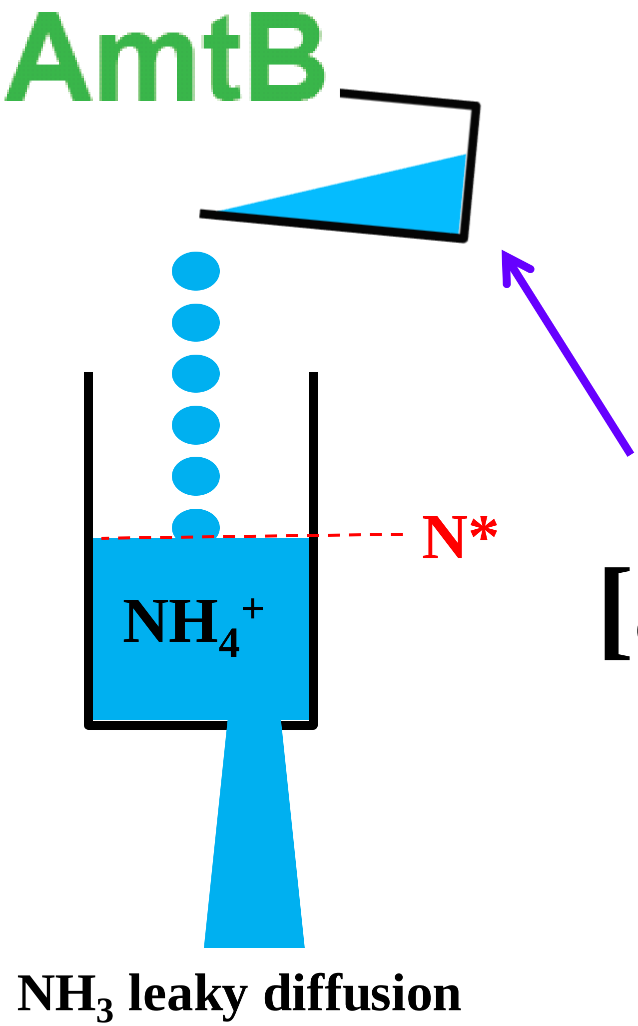

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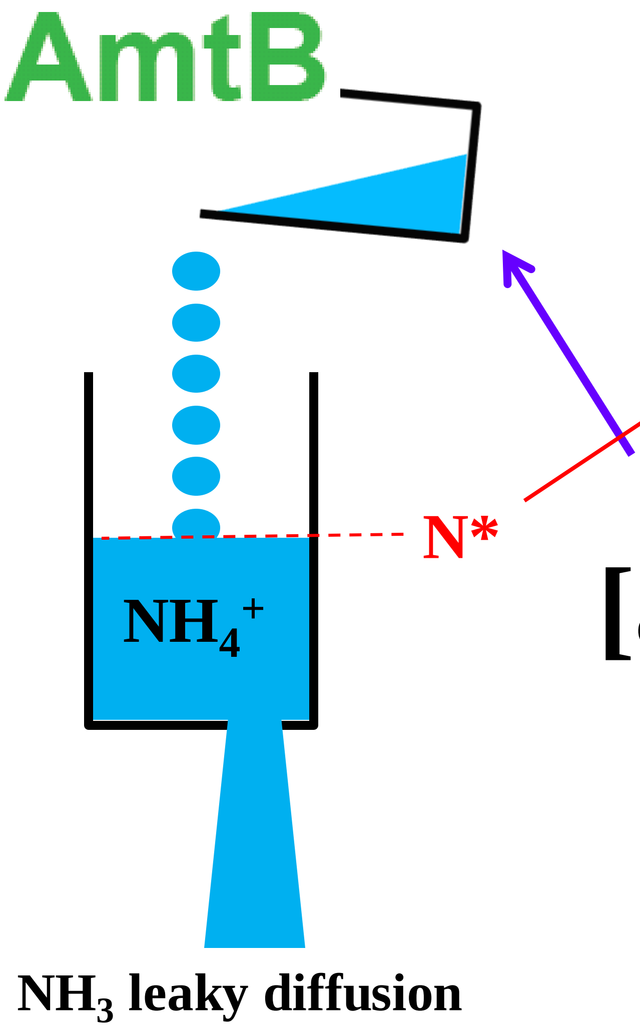

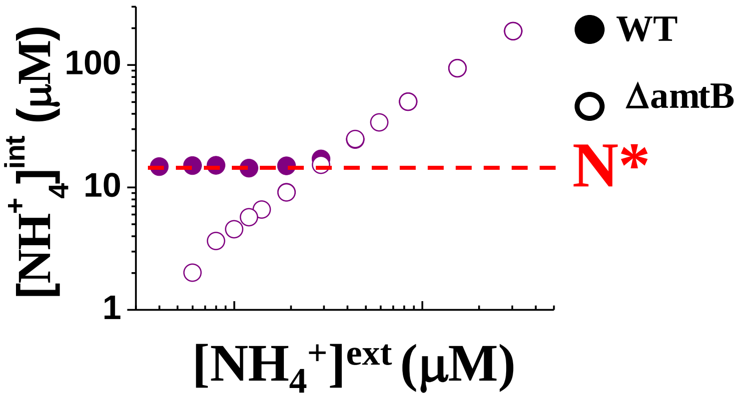

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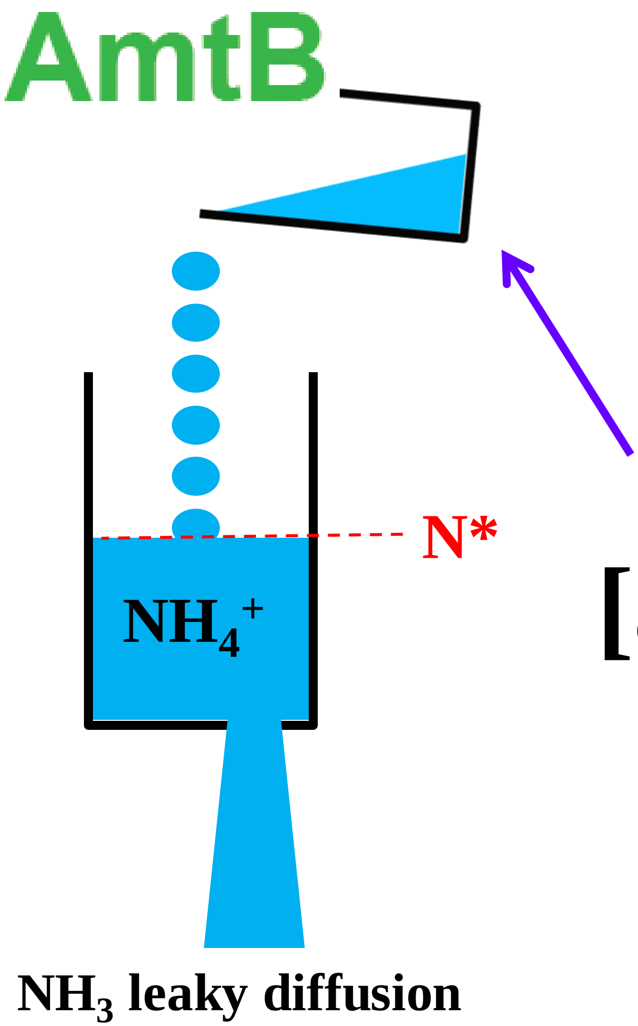
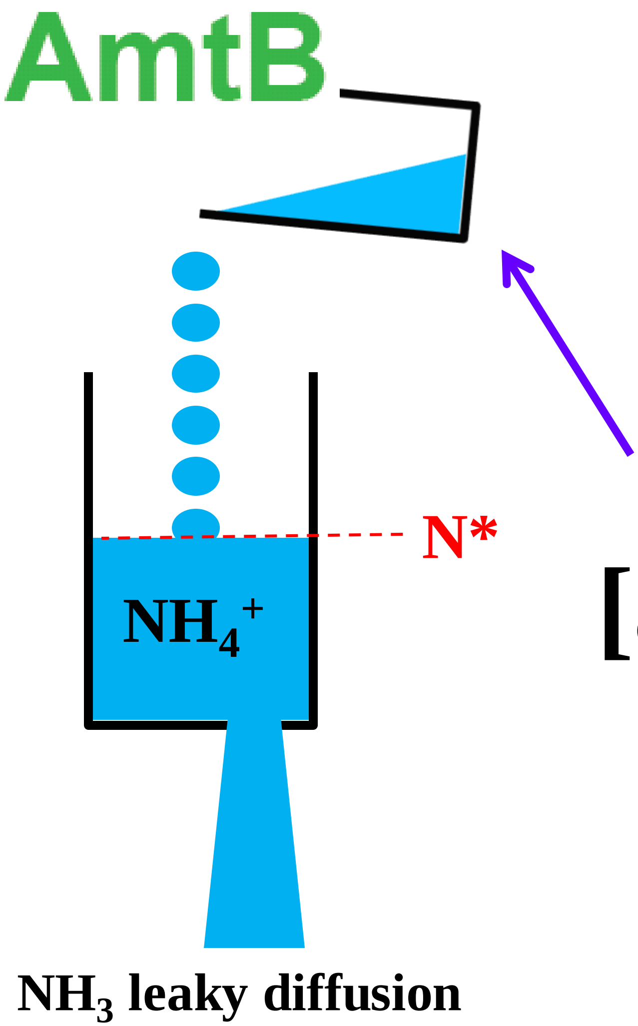
Negative integral feedback

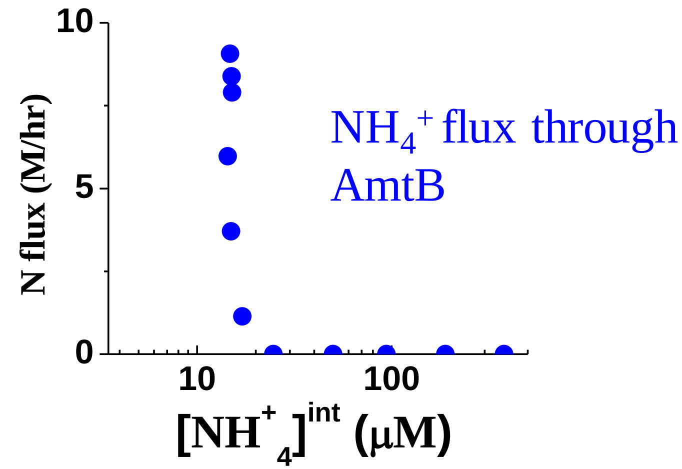


$$[aKG] = \int J_{\text{biomass}} - J_{\text{N assimilation}} \, dt$$

Amplify signal

Negative integral feedback





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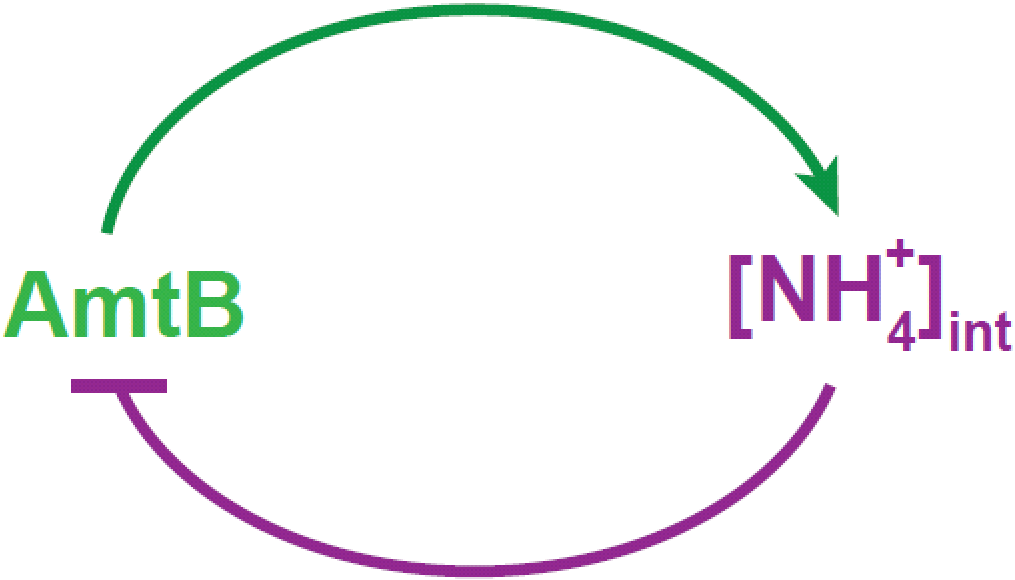
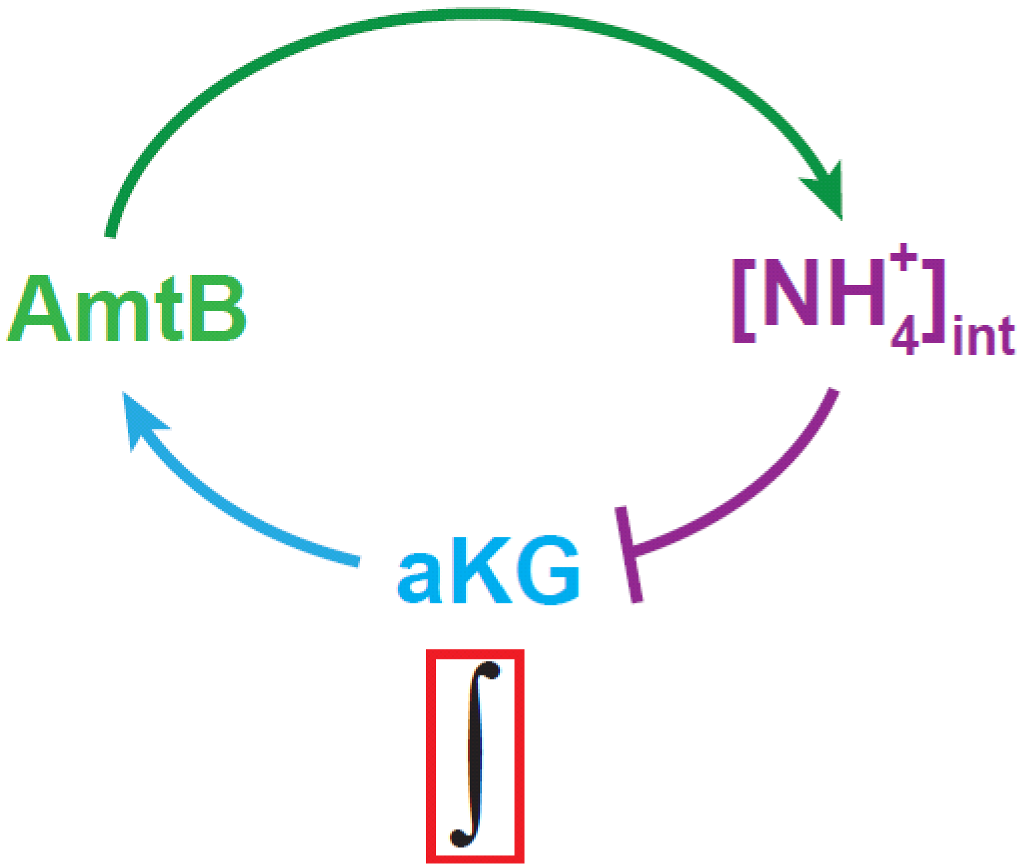


Negative integral feedback



Integral feedback control

- Integral feedback control : Common engineering scheme that allows a system to track a desired set-point robustly.
  - e.g. thermostat in the room, cruse control in a car.

- AmtB is regulated very <u>sensitively</u> so that internal ammonium is maintained <u>precisely</u> at the minimal level needed for the optimal growth.

Kim, M. *et al.* (2012) *Mol. Syst. Biol*. 8:616

Tribute to Sydney Kustu