

Abstract Submitted
for the MAR11 Meeting of
The American Physical Society

Steady state growth of *E. Coli* in low ammonium environment

MINSU KIM, BARRET DERIS, ZHONGGE ZHANG, TERRY HWA, UCSD — Ammonium is the preferred nitrogen source for many microorganisms. In medium with low ammonium concentrations, enteric bacteria turn on the nitrogen responsive (*ntr*) genes to assimilate ammonium. Two proteins in *E. coli*, Glutamine synthetase (GS) and the Ammonium/methylammonium transporter AmtB play crucial roles in this regard. GS is the major ammonium assimilation enzyme below 1mM of NH_4^+ . AmtB is an inner membrane protein that transports NH_4^+ across the cell membrane against a concentration gradient. In order to study ammonium uptake at low NH_4^+ concentration at neutral pH, we developed a microfluidic flow chamber that maintains a homogenous nutrient environment during the course of exponential cell growth, even at very low concentration of nutrients. Cell growth can be accurately monitored using time-lapse microscopy. We followed steady state growth down to micro-molar range of NH_4^+ for the wild type and ΔamtB strains. The wild type strain is able to maintain the growth rate from 10mM down to a few μM of NH_4^+ , while the mutant exhibited reduced growth below $\sim 20 \mu\text{M}$ of NH_4^+ . Simultaneous characterization of the expression levels of GS and AmtB using fluorescence reporters reveals that AmtB is turned on already at 1mM, but contributes to function only below $\sim 30 \mu\text{M}$ in the wild-type. Down to $\sim 20 \mu\text{M}$ of NH_4^+ , *E. coli* can compensate the loss of AmtB by GS alone.

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Date submitted: 27 Dec 2010

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