187. A Computational Model of Methane Producing Archaea

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Project Goals: Methanogens utilize a wide variety of substrates to produce methane, which has implications for bioenergy production and global carbon cycling. This project's main goal is the development of a whole-cell model for making predictions about the Methanogenesis pathways in members of the *Methanosarcina* genus. This entails the integration of a stochastic kinetic model of the methanogenic pathways with a transcriptionally regulated metabolic model with which the environmental conditions that induce population heterogeneity may be identified. We will model the response of *M. acetivorans* (marine) and *M. barkeri* (freshwater) to environmental fluctuations. The modeling leverages the expertise of the C. Woese and W. Metcalf labs on the genetics and evolution of the methanogens, as well as single molecule and biochemical experiments. The integration of kinetics with genome-scale metabolic/regulatory models constitutes a novel methodology for systems biology studies of cellular phenotypes.

Progress towards a more complete model of the methanogenic archaeum *Methanosarcina acetivorans* is reported [1]. We characterized size distribution of the cells using differential interference contrast (DIC) microscopy, finding them to be ellipsoidal with mean length and width of 2.9 µm and 2.3 µm respectively when grown on methanol, and on average 2.3 µm long and 1.7 µm nwide when grown on acetate (Figure 1). We used the single molecule pull down (SiMPull) technique to measure average copy number of the Mcr complex and ribosomes (Table 1). In creating a model, RNA expression data (RNA-seq) measured for cell cultures grown on methanol and trimethylamine (TMA) can be used to estimate relative protein production per mole of ATP consumed. A kinetic model for the methanogenesis pathways based on biochemical studies that have been further validated by recent metabolic reconstructions for several related methanogens, is presented (Figure 2a). The kinetic model is capable of capturing experimentally observed mean methane production and mean substrate consumption rates for cell cultures growing on methanol and TMA/methanol mixtures (Figure 2b,c) In this model, twenty-six reactions in the methanogenesis pathways are coupled to a cell mass production reaction that updates enzyme concentrations. The archaeum's growth was most sensitive to the number of methyl-coenzyme- M reductase (Mcr) and methyl-

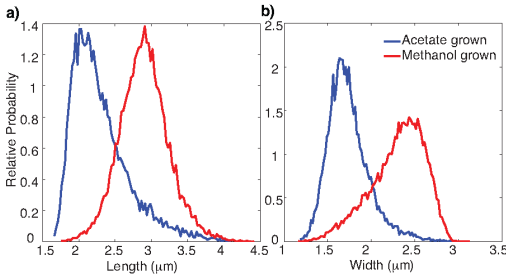

Figure 1 Distributions of (a) length and (b) width of *M. acetivorans* cells grown in methanol and acetate. Methanol grown cells are on average 9 fL and about 2.2x larger than those grown in acetate.

Biological Replicate	Protein Copy/Cell
Mcr	
1	320±713
2	273±124
Rpl18	
1	10038±3340
2	18135±6040

Table 1 Measured copy number per cell (grown in methanol) of two highly expressed proteins

tetrahydromethanopterin: coenzyme-M methyltransferase (Mtr) proteins. A draft model of transcriptional regulation based on known interactions is proposed which we intend to integrate with the kinetic model to allow dynamic regulation (Figure 3).

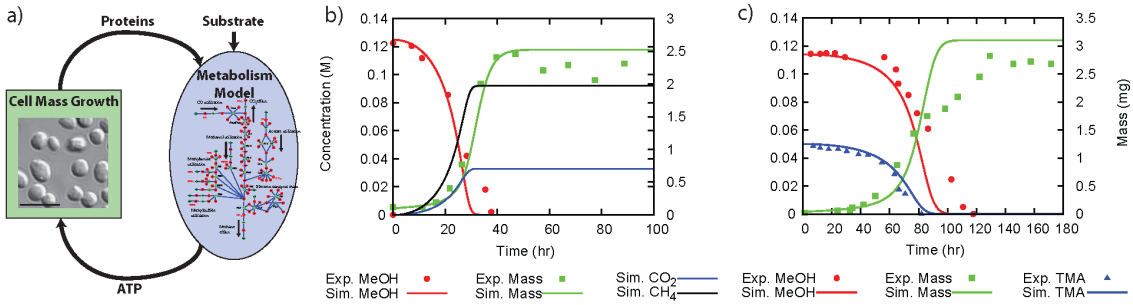

Figure 2 a) Kinetic model for the methanogenesis pathway is coupled to a cell culture mass growth reaction to simulate colony growth. Relative protein production ratios are based on RNA expression data. b) Simulated colony growth on 125 mM methanol for 100 hours showing good agreement to experiments [2]. c) Simulated colony growth on 125 mM methanol and 50 mM TMA for 180 hours also showing good agreement to experiments [2].

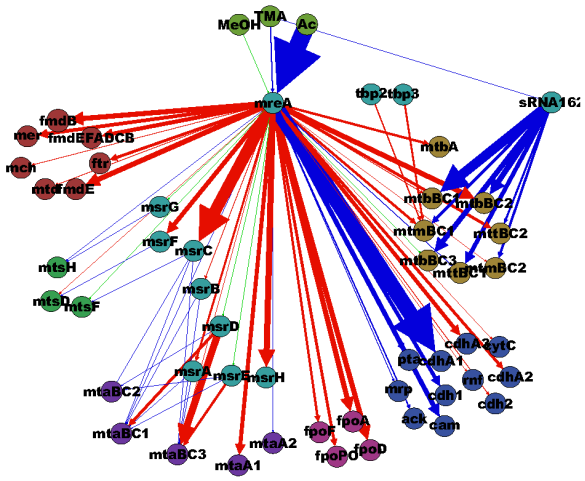

Figure 3A regulation model based on transcription factors (cyan) that promote (blue arrows) or repress (red arrows) expression of different methanogenesis proteins. mreA, turns off many of the methylotrophic proteins and turns on manyMethylamine e into the cells as well as proteins that fix nitrogen, in the presence of MMA, DMA or TMA

References

1. J.R. Peterson, P. Labhsetwar, J.R. Ellermeier, P.R.A. Kohler, A. Jain, T. Ha, W.W. Metcalf, Luthey-Schulten. *Archaea*, 2014, *In Press*.
2. Bose, M. Pritchett, M. Rother, and W. Metcalf. *Journal of Bacteriology*, vol. 188, no. 20, pp. 7274–7283, 2006.

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