Molecular crowding represents a physical constraint that regulates cell growth through metabolic pathway selection

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Previously, a flux balance-based modeling framework that include molecular crowding (MC) as a constraint, accurately predicted substrate uptake sequence in *E. coli* cells cultured in mixed substrate. This strongly indicates that MC is a crucial constraint for cell metabolism, especially in fast growing cells which predominantly use glycolysis pathway. Here we aim to study how MC modulation affects cell growth in *E. coli*. By studying cell growth and morphology of strains with different MC levels, we find that glycolytic cells prefer higher MC environment, and optimal intracellular MC is a necessary condition for rapid cell growth.

***Keywords* — Molecular crowding (MC), glycolysis, mixed substrate culture**

I. Purpose

The dynamic reorganization of cellular metabolism according to the available carbon sources is crucial for bacterial survival and thriving in fluctuating environments. Yet, the underlying physicochemical attributes of the cell that constrain its achievable metabolic states remains only partially understood. In our previous studies, we generated indirect evidence of the role of MC in constraining cellular physiology. Incorporating MC into a flux-balance analysis (FBA-MC) successfully predicted the orders of substrate uptake of *E. coli* cells grown in mixed substrate media (*1*). FBA-MC also successfully predicts changes in intracellular metabolic flux distribution where MC is indirectly influenced by changing the growth rate of *E. coli* cells (*2*). In addition, incorporation of MC into a yeast glycolysis kinetic model successfully predicts intracellular metabolite levels in *S. cerevisiae* (*3*).

Here we aim to directly test how MC regulates cell growth especially for fast growing cells that utilize the glycolysis pathway usage in *E. coli*. We use gene deletion strains and cell morphology mutants, which have different intracellular MC levels to study MC effects on cell growth. First, we studied cell growth in a genome reduced *E. coli* strain, finding that higher MC cells with smaller cell volume grew faster than the wild type control in glycolytic substrates but not in non-glycolytic substrate. This indicates that fast growing cells, which are glycolytic, may benefit from high MC environment or they may be more sensitive to MC fluctuation. The high MC environment hindered cell growth in mixed substrate culture, which implies that CCR (carbon catabolite repression) mechanism may be the intrinsic stabilizer for optimal intracellular MC for *E. coli* cells. We also directly manipulated intracellular MC by overexpressing an exogenous protein under IPTG induction. Intracellular MC was significantly increased with minor growth retardation in glucose-limited culture. We are also testing cell growth of an *E. coli* cell shape mutant with altered MC level in different substrates cultures. The results will provide information about the correlation between morphology and MC stability. The associated cell density and cell volume changes along the growth also imply that cells tend to adjust the intracellular MC to achieve efficient growth.

II. Conclusion

MC is a critical constraint for cell physiology. Cells fine tune MC and associated cell size to adapt to the changing environment and the available resources. As a result, the proper metabolism rate is determined and contributes to the optimal cell growth at the given condition (*4, 5*). The growth phenotypes observed in *E. coli* cells with different MC levels will shed light on glycolysis pathway selection in fast growing cells.

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