Intracellular crowding of macromolecules defines the mode and sequence of substrate uptake by *E. coli* and constrains its metabolic activity and growth

Supporting Information, Part 3: Supplementary Tables

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Table S2: Full names of genes, enzymes their functions and substrates shown in Figure 3

Table S3: (separate excel table-Supporting file 5): Time series microarray data of cultures shown in Figure 2, 3 and 4

Table S4a: (separate excel table-Supporting file 6): Microarray data of single carbon-limited batch-cultures, described in Figure 4 (bottom panel)

Table S4b: (separate excel table-Supporting file 7): Microarray data of glucose and lactate-limited batch-cultures, described in Figure 4 (bottom panel)

Supplementary Tables

Table S2: Major functions and reactions catalyzed by the transporters/enzymes encoded by the genes involved in substrate uptake, as shown in Figure 3 of the manuscript

Gene and carbon source	Functions and reactions catalyzed by enzymes/proteins
Glucose	
ptsG	Functions: The product of *ptsG* gene (glucose-specific PTS permease) is responsible for uptake of exogenous glucose from the medium, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism, primarily via glycolysis (Buhr and Erni 1993; Postma et al. 1993). PtsG/Crr, the glucose-specific PTS permease, belongs to the functional superfamily of the phosphoenolpyruvate (PEP)-dependent, sugar transporting phosphotransferase system (PTS). The PTS transports and simultaneously phosphorylates its sugar substrates in a process called group translocation. Reaction: phosphoenolpyruvate + β-D-glucose[periplasmic space] → β-D-glucose-6-phosphate + pyruvate
Maltose	
malEFGK	Functions: *malKFGE* operon plays the major role in the maltose transport system and it belongs to the ATP-Binding Cassette (ABC) superfamily of transporters (Wu and Mandrand-Berthelot 1995). *malE* is the periplasmic maltose-binding protein, *malF* and *malG* are the integral membrane components of the ABC transporter, and *malK* is the ATP-binding component of the ABC transporter ($MalFGK_2$) (Boos and Shuman 1998; Joly et al. 2004) Reactions: $\text{maltose}_{[\text{extracellular space}]} \Leftrightarrow \text{maltose}_{[\text{cytosol}]}$ $\text{ATP} + \text{maltose}_{[\text{periplasmic space}]} + H_2O \Leftrightarrow \text{ADP} + \text{phosphate} + \text{maltose}_{[\text{cytosol}]}$
malQ	Functions: *malQ* codes for amylomaltase, which is responsible for degrading maltose after transport into the cell (Neidhardt et al. 1996). The glucose liberated in the degradation reaction is then used in glycolysis. Amylomaltase also recognizes maltotriose and larger maltodextrins (donors), cleaving off the reducing glucose residue and transferring the remaining dextrinyl residue onto the nonreducing end of maltodextrin (acceptors), including maltose and glucose. Amylomaltase thus produces glucose and longer maltodextrins from maltotriose, the smallest donor substrate, as well as from longer linear maltodextrins (Boos and Shuman 1998) Reaction:

	H_2O + maltose <=> 2 β-D-glucose maltotriose + maltose <=> maltotetraose + β-D-glucose
glk	Functions: Under normal conditions, glucokinase plays a minor role in *E. coli* glucose metabolism. Under anabolic stress the enzyme is required to supplement the levels of glucose 6-phosphate. Reactions: β-D-glucose + ATP <=> β-D-glucose-6-phosphate + ADP
Galactose	
mglABC	Functions: MglABC is a beta-methylgalactoside transport system that is a member of the ATP-Binding Cassette (ABC) superfamily of transporters (Wu and Mandrand-Berthelot 1995). The *mglB* gene codes for a galactose-binding protein that serves both as the galactose chemoreceptor as well as the recognition component of the β-methylgalactoside transport system, which utilizes the galactose-binding protein; *mglC* encodes the integral membrane component; and *mglA* encodes the ATP-binding component of the ABC transporter (Harayama et al. 1983). Reaction: ATP + β-D-galactose$_{[\text{periplasmic space}]}$ + H_2O $\Leftrightarrow$ ADP + phosphate + β-D-galactose$_{[\text{cytosol}]}$
galE	Functions: *galE* codes for UDP-galactose 4-epimerase, which catalyzes a hydride transfer and the interconversion of UDP-galactose and UDP-glucose as part of galactose catabolism (Swanson and Frey 1993). Reaction: UDP-D-glucose <=> UDP-galactose
galK	Functions: Galactokinase, coded by *galK* catalyzes the first step in the galactose metabolism Reaction: D-galactose + ATP = α-D-galactose 1-phosphate + ADP
galT	Functions: *galT* codes for galactose 1-phosphate uridylyltransferase, which catalyzes an interconversion reaction in galactose catabolism Reaction: UDP-D-glucose + α-D-galactose 1-phosphate = α-D-glucose 1-phosphate + UDP-galactose α-D-galactose 1-phosphate + UTP = UDP-galactose + diphosphate
galP	Functions:

	GalP is one of two, along with MglABC, major routes for galactose transport into *E. coli*. 2-deoxy- D-galactose is a specific substrate for GalP but not for MglABC and GalP operates by a sugar-proton symport mechanism while MglABC does not. Reaction: $H^+_{[periplasmic\ space]}$ + β-D-galactose$_{[periplasmic\ space]}$ ⇔ $H^+_{[cytosol]}$ + β-D-galactose$_{[cytosol]}$
pgm	Functions: *pgm* codes for phosphoglucose mutase, which catalyzes conversion of glucose-1-phosphate to glucose-6-phosphate. Maximum activity is only obtained in the presence of α-D-glucose 1,6-bisphosphate. This bisphosphate is an intermediate in the reaction, being formed by transfer of a phosphate residue from the enzyme to the substrate, but the dissociation of bisphosphate from the enzyme complex is much slower than the overall isomerization. Reaction: α-D-glucose-1-phosphate → α-D-glucose-6-phosphate
Glycerol	
glpK	Function: *glpK* codes for glycerol kinase, which catalyzes the MgATP-dependent phosphorylation of glycerol to yield sn-glycerol 3-phosphate (Pattigrew et al. 1988). This is also the rate limiting step in glycerol utilization in *E. coli*. Reaction: glycerol + ATP → *sn*-glycerol-3-phosphate + ADP
glpF	Function: The glycerol facilitator, GlpF, allows the facilitated diffusion of glycerol into the cell (Stroud et al. 2003). Reaction: glycerol$_{[periplasmic\ space]}$ ⇔ glycerol$_{[cytosol]}$
gpsA	Functions: *gpsA* codes for glycerol-3-phosphate-dehydrogenase-[NAD(P)$^+$], which catalyzes the NAD(P)H-dependent reduction of the glycolytic intermediate dihydroxyacetone-phosphate to produce glycerol-3-phosphate (Lin 1976) Reaction: *sn*-glycerol-3-phosphate + NAD(P)$^+$ = dihydroxyacetone phosphate + NAD(P)H + H^+
Lactate	
lldP	Function: LldP (or LctP) is a lactate/proton symporter responsible for the uptake of L-lactate. The *lldP/lctP* gene is located in a lactate-inducible operon with the *lctD* and *lctR* genes encoding a lactate dehydrogenase and a regulatory protein, respectively (Dong et al. 1993). Reaction: $H^+_{[periplasmic\ space]}$ + lactate$_{[periplasmic\ space]}$ ⇔ $H^+_{[cytosol]}$ + lactate$_{[cytosol]}$

dld	Function: *dld* codes for D-lactate dehydrogenase. There are three lactate dehydrogenase enzymes in *E. coli* which interconvert pyruvate and lactate. One is an NAD-linked fermentative dehydrogenase. The other two are membrane-bound flavoproteins, each specific for the D- or L-isomer, and involved in the aerobic respiratory chain of *E. coli*. The D-lactate dehydrogenase is coded for by the *dld* gene and it is the primary source of energy to drive the active transport of certain sugars and amino acids into the cell (Matsushita et al. 1986). Reaction: ubiquinone-*8* + D-lactate ⇔ ubiquinol-*8* + pyruvate
Acetate	
ackA	Function: The *ackA* gene product has propionate kinase activity as well as acetate kinase activity. It is unclear whether the two *ack* genes, *ackA* and *ackB*, code for two distinct acetate kinase enzymes or control a single enzyme. Helps in conversion of acetate to acetyl phpsphate. The *ackA* encoded propionate kinase 2 has an important role in propionyl-CoA metabolism (Hesslinger et al. 1998). Acetate kinase can also catalyze acetylation of CheY, increasing signal strength for flagellar rotation. (Barak et al. 1998) Reactions: ATP + propionate ⇔ ADP + propionyl-P acetate + ATP ⇔ acetylphosphate + ADP
pta	Function: *pta* gene codes for phosphate acetyltransferase, which can utilize both acetyl-CoA and propionyl-CoA. Reactions: phosphate + acetyl-CoA ⇔ acetylphosphate + coenzyme A propionyl-CoA + phosphate ⇔ propionyl-P + coenzyme A
acs	Function: *acs* gene codes for acetyl-CoA synthetase. There are two distinct pathways by which *E. coli* converts acetate to acetyl-CoA. Acetyl-CoA synthetase catalyzes one of them. It is thought that this ACS pathway functions in a mainly anabolic role, scavenging acetate present in the extracellular medium (Kumari et al. 1995). ACS also can catalyze acetylation of CheY, increasing signal strength for flagellar rotation (Ramakrishna et al. 1998). Reactions: coenzyme A + 4-coumarate + ATP → coumaroyl-CoA + diphosphate + AMP coenzyme A + propionate + ATP ⇔ propionyl-CoA + diphosphate + AMP coenzyme A + acetate + ATP ⇔acetyl-CoA + diphosphate + AMP

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