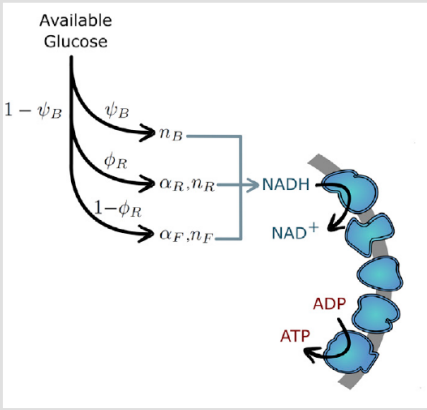
Box 1. Membrane Requirements of ATP Production

Respiration and acetate fermentation are the two major routes to ATP generation in *E. coli* grown aerobically on glucose (Neidhardt and Curtiss, 1996). While *E. coli* can perform several different kinds of fermentation, acetate is the predominant end product of overflow metabolism in glucose media (Valgepea et al., 2011; O'Brien et al., 2013). ATP generation by both routes can be divided into electron transport chain-dependent and electron transport chain-independent parts. This is shown in the Figure B1, where α represents the number of ATP produced independently of the electron transport chain, while n represents the number of NADH equivalents produced that are used by the electron transport chain to generate ATP (Table S1C).



	NDH-I	SDH	Cyo	Cyd-I	ATP synthase
Stoichiometry	1/4	1	1/3	1/4	1
Membrane area (nm^2)	80	10	35	30	35
Per respiratory unit (nm^2)	20	10	12	7	35

* NDH-I = NADH dehydrogenase I; SDH = succinate dehydrogenase; Cyo = cytochrome *bo* terminal oxidase; Cyd-I = cytochrome *bd*-I terminal oxidase.

ATP source	ATP rate (ATP/s)	Membrane area (nm^2)	Surface efficiency (ATP/s/nm^2)
ETC-independent	720	48	15
ETC-dependent	270	84	3

Metabolic mode	ETC-independent surface efficiency (ATP/s/nm^2)	ETC-dependent surface efficiency (ATP/s/nm^2)	Total surface efficiency (ATP/s/nm^2)
Acetate Fermentation	15	-	15
Respiration	15	3	4

Figure B1. Acetate Fermentation Is More Surface-Efficient than Respiration

(Left) NADH and ATP production depends on how glucose is partitioned between biomass production, acetate fermentation, and respiration. NADH is consumed by the electron transport chain to produce ATP. Top right: membrane real estate occupied per electron transport chain complex and per respiratory unit, normalizing to ATP synthase. Middle right: surface efficiency of electron transport chain (ETC)-independent energy generation (transporter + cytosolic processes) and ETC-dependent processes (only ETC). Bottom right: the net surface efficiency of each metabolic mode depends on its component efficiencies. Our calculations assume a phosphate/oxygen (*P/O*) ratio of 1.0 (Hempfling and Mainzer, 1975; Farmer and Jones, 1976; Noguchi et al., 2004; Feist et al., 2007) for conversion of NADH to ATP via the electron transport chain (Tables S1A and S1B).

For each part, we can define surface efficiency as the number of ATP/s produced from a given amount of membrane area. The electron transport chain-independent part requires only glucose transporters (48 ± 10 nm^2/dimer), which can supply a maximum of 180 ± 40 glucose/s that each yield 4 ATP in both respiration and acetate fermentation. This gives a surface efficiency of 15 ± 5 ATP/s/nm^2. The surface efficiency of the electron transport chain-dependent part can be estimated from experimental electron transport chain protein structures, abundances, and kinetics (Valgepea et al., 2013; Etzold et al., 1997). Normalizing abundances to the terminal ATP synthase, an estimated 84 nm^2 of electron transport chain is required to generate 270 ± 40 ATP/s, the maximum speed of ATP synthase (Etzold et al., 1997), resulting in a surface efficiency of only 3 ± 1 ATP/s/nm^2 (Tables S1B and S1D). The electron transport chain stoichiometry shown in the table below represents averages across growth rates (Valgepea et al., 2013) (Table S1B).

A key conclusion from these calculations is that the larger number of NADH equivalents produced from acetyl-CoA by the TCA cycle (7.2 , Table S1C [Alberts et al., 2002]) compared with acetate fermentation (0) is both the source of respiration's greater ATP yield *and* its lower surface efficiency, as it creates a high demand for surface-inefficient electron transport chain. It therefore follows that once the inner membrane reaches its protein packing limit, the cell can continue to increase its ATP production rate and growth rate by gradually increasing the fraction of glucose directed to acetate fermentation rather than the TCA cycle, as observed experimentally (Vemuri et al., 2006; Valgepea et al., 2011; O'Brien et al., 2013).

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). Molecular Biology of the Cell, Fourth Edition (Garland Science).

Etzold, C., DeckersHebestreit, G., and Altendorf, K. (1997). Turnover number of *Escherichia coli* f0f1 ATP synthase for ATP synthesis in membrane vesicles. Eur. J. Biochem. *243*, 336–343.

Farmer, I.S., and Jones, C.W. (1976). Energetics of Escherichia-coli during aerobic growth in continuous culture. Eur. J. Biochem. *67*, 115–122.

Feist, A.M., Henry, C.S., Reed, J.L., Krummenacker, M., Joyce, A.R., Karp, P.D., Broadbelt, L.J., Hatzimanikatis, V., and Palsson, B.Ø. (2007). A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. Mol. Syst. Biol. *3*, 121.

Hempfling, W.P., and Mainzer, S.E. (1975). Effects of varying the carbon source limiting growth on yield and maintenance characteristics of *Escherichia coli* in continuous culture. J. Bacteriol. *123*, 1076–1087.

Neidhardt, F.C., and Curtiss, R. (1996). *Escherichia coli* and Salmonella: Cellular and Molecular Biology, Second Edition (ASM Press).

Noguchi, Y., Nakai, Y., Shimba, N., Toyosaki, H., Kawahara, Y., Sugimoto, S., and Suzuki, E. (2004). The energetic conversion competence of *Escherichia coli* during aerobic respiration studied by 31P NMR using a circulating fermentation system. J. Biochem. *136*, 509–515.

O'Brien, E.J., Lerman, J.A., Chang, R.L., Hyduke, D.R., and Palsson, B.O. (2013). Genome-scale models of metabolism and gene expression extend and refine growth phenotype prediction. Mol. Syst. Biol. *9*, 693.

Valgepea, K., Adamberg, K., and Vilu, R. (2011). Decrease of energy spilling in *Escherichia coli* continuous cultures with rising specific growth rate and carbon wasting. BMC Syst. Biol. *5*, 106.

Valgepea, K., Adamberg, K., Seiman, A., and Vilu, R. (2013). *Escherichia coli* achieves faster growth by increasing catalytic and translation rates of proteins. Mol. Biosyst. *9*, 2344–2358.

Vemuri, G.N., Altman, E., Sangurdekar, D.P., Khodursky, A.B., and Eiteman, M.A. (2006). Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. Appl. Environ. Microbiol. *72*, 3653–3661.