Table 1 Measured and predicted parameters for *E. coli* growing on minimal medium

Metabolite	Pool size (μmol g_{CDW}^{-1})[a]	k (min^{-1})[a,b]	Measured flux (KFP) (mmol g_{CDW}^{-1} hr^{-1})[c]	Predicted flux (FBA) (mmol g_{CDW}^{-1} hr^{-1})[c]
Glutamine (**1**)	3.92 ± 0.17	14.29 ± 6.28	3.36	0.88
Glutamate (**2**)	100.55 ± 17.54	0.79 ± 0.04	4.77	4.61
Alanine (**4**)	6.81 ± 1.70	≥1.6 ± 0.38	≥0.65	0.26
Asparagine (**5**)	2.02 ± 0.46	3.06 ± 0.71	0.37	0.11
Aspartate (**6**)	6.45 ± 3.54	2.88 ± 0.25	1.12	1.08
Methionine (**7**)	0.29 ± 0.07	≥1.47 ± 0.33	≥0.025	0.071
Phenylalanine (**8**)	0.20 ± 0.03	5.12 ± 1.62	0.063	0.081
Proline (**9**)	1.10 ± 0.15	≥3.02 ± 0.66	≥0.20	0.097
Threonine (**10**)	1.34 ± 0.16	7.52 ± 2.71	0.61	0.24
Tyrosine (**11**)	0.41 ± 0.25	9.51 ± 7.73	0.23	0.061
Valine (**12**)	2.41 ± 0.27	3.88 ± 1.14	0.56	0.19
Carbamoyl aspartate (**13**)	0.84 ± 0.28	4.11 ± 0.98	0.21	0.15
IMP (**14**)	0.38 ± 0.01	≥2.83 ± 0.27	≥0.064	0.26

[a]Error estimates for pool size are s.d. of quadruplicate measurements; error estimates for k are standard errors from the curve fit. [b]k = apparent first-order rate constant for ^{15}N labeling; see **Supplementary Methods**, equation (8). [c]KFP measures gross fluxes, whereas FBA predicts net fluxes. g_{CDW} = grams of cell dry weight. IMP, inosine monophosphate.

loaded with minimal essential medium. Nutrients diffused readily up through the agarose and filters to the *E. coli*, which grew exponentially with a doubling time similar to that obtained in comparable liquid medium (75 min versus 87 min; **Fig. 1a**). The *E. coli*–laden filters can be transferred from one agarose plate to another, thereby enabling nondisruptive, fast modification of the nutrient environment.

The kinetics of ammonia diffusion from an agarose plate onto a blank filter are shown in **Figure 1b**. Also shown in **Figure 1b** are the labeling kinetics of the central-nitrogen-metabolism compounds glutamine (**1**) and glutamate (**2**), following transfer of an *E. coli*–laden filter from normal minimal medium to minimal medium containing [^{15}N]ammonia. Labeling of the amide nitrogen of glutamine, which results from addition of ammonia to glutamate at the expense of ATP (**Fig. 1c**), occurred slightly less rapidly than the limit implied by the ammonia diffusion rate (**Fig. 1b**), resulting in a large rate constant k_Q for turnover of the glutamine amide nitrogen (**Table 1**).

Glutamate is present in enteric bacteria in amounts $\sim$25-fold larger than those of glutamine (**Table 1**)[12]. Glutamate can be synthesized either directly from ammonia via the glutamate dehydrogenase system or indirectly via glutamine and glutamate synthetase (**Fig. 1c**). We found that glutamate becomes labeled less rapidly than glutamine ($k_E << k_Q$, where k_E is the rate constant for turnover of glutamate's nitrogen), but the glutamate flux is nevertheless slightly greater than that of glutamine owing to the much larger glutamate pool size (**Table 1**). Production of double-labeled glutamine precisely mirrored labeling of glutamate, which provides glutamine's amino nitrogen (**Fig. 1d**). This observation is consistent with the notion that glutamate serves as the parent of doubly labeled glutamine, with $k_Q >> k_E$.

Nitrogen is funneled from glutamine and glutamate into nucleotides and amino acids[13] (**Supplementary Fig. 1** online). We found that nucleotide biosynthetic intermediates become labeled very rapidly, consistent with their being products of glutamine that are present in relatively low amounts but sit on high-flux pathways. Because most amino acids receive nitrogen from glutamate, they became labeled very shortly after glutamate, consistent with the large pool size of glutamate relative to its amino acid products.

The flux measurements obtained here can be compared to the predictions of FBA, assuming that *E. coli* grown under the present conditions are optimizing their growth rate per molecule of glucose consumed (**Table 1**). The most important conceptual difference

between the FBA predictions and our results is that FBA predicts net flux (that is, for reversible reactions, forward flux – reverse flux), whereas the present observations are of gross flux (that is, forward flux). With this caveat in mind, the overall agreement between the measured and predicted values is reasonable, especially for irreversible reactions (for example, glutamate synthesis and pyrimidine biosynthesis). The most notable discrepancy between the measured fluxes and those predicted by FBA occurs for glutamine and arises from assuming, in the optimization step of FBA, maximization of growth yield per glucose molecule. Our data show that when adequate glucose is available, even with ample extracellular ammonia present, ATP is spent to synthesize glutamate largely via glutamine, despite the presence of the alternative, lower-energy-cost pathway of glutamate dehydrogenase[14]. The observed glutamine flux is $\sim$400% of that expected if no glutamate were made via glutamine, and $\sim$60% of that expected if all glutamate were.

The experiments conducted here provide support for both the predictive power of FBA and the measurement capabilities of KFP. They support a picture of nitrogen assimilation[12] in *E. coli* in which glutamine is a short-lived intermediate in the synthesis of glutamate, the main warehouse of free nitrogen in enteric bacteria. Because the time between isotope switching and collection of critical nitrogen flux data is short ($\sim$5 min), KFP should be able to provide insight into the changes in flux patterns that occur in response to environmental perturbations. Preliminary data supporting this, obtained using the example of fast turnoff of biosynthetic fluxes during carbon starvation, are presented in **Supplementary Figure 2** online. Future studies exploring the applicability of KFP to other nutrients, organisms and experimental conditions are warranted.

Note: Supplementary information is available on the Nature Chemical Biology website.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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