**Figure 1** **Overview of experimental work flow.**

A) Measurements of RNA, protein, lipids, and metabolic flux are taken under uniform growth and environmental conditions. B) Long-term glucose starvation experiment. E. coli REL606 strain was taken from freezer stock and revived in 10ml DM500 media with minimal glucose for 24 hrs (day -2). The next day this culture was diluted 1:10 in 500mL DM500 and grown for 24 hrs(day-1). On the day of the experiment (day 0) the previously conditioned culture was diluted again into several individual cultures. C) The OD @ 600 was measured to asses growth and optimal collection of time points. Nine time points where selected for this experiment spanning 3hr-2wks. D) For each sample 1ml was removed from the culture for each experiment to be done, spun down, flash frozen, and distributed to the experimental labs in our consortium to measure RNA via RNA seq, protein via LC/MS, lipids via Maldi-TOF MS and ESI MS, and metabolic flux via GC-MS.

**Figure 2 K-means clustering of RNA and protein profiles in long-term glucose starvation**

RNA and Protein profiles, normalized to each molecules maximum value, where clustered by geometric distance using K-means clustering with 15 and 25 clusters respectively. The cluster centroids where then plotted as heat maps with darker blue representing higher RNA, or protein, levels. A) RNA levels are largely shutoff upon glucose starvation with some RNA being temporarily up regulated during the transition between exponential and stationary growth. B) Protein levels show a much wider range of behaviors with some being up or down regulated for the duration of the experiment as well as for a short period of time during the transition from exponential to stationary growth. C) Histogram of the correlation coefficients between individual protein levels and the time integral of RNA. This is the limit of slow protein degradation where protein levels are proportional to the cumulative sum of their respective transcripts. D) Histogram of the correlation coefficient between relative protein levels and their corresponding (relative) transcripts. This is a measure of how proportional proteins are to their respective RNAs. E) 2-D histogram of the correlation coefficient of protein vs. RNA (on the x-axis) and the protein vs. the time integral of RNA (y-axis). Darker colors indicate more genes in that given bin. There is a strong anti-correlation between the two measures of dynamic correlation indicating that these two quantities are largely mutually exclusive. F) The correlation between all RNA and protein levels for a single time point is weak ( Spearman correlation coefficient of 0.59).

**Figure 3 Intra operon correlation.**

(A) and (B) histograms of the median pairwise correlation coefficient between all possible pairs of RNA and Protein profiles, respectively, within an operon. Proteins that have a smaller inter-gene distance are more likely to have correlated profiles ( C). 2D Histogram of the pairwise correlation between proteins in the same operon, y-axis, and the inter-gene distance between the protein coding regions, x-axis.

(D) and (E) Examples of proteins in the same operon that are highly correlated. (F) and (G) examples of RNAs in the same operon that are highly correlated.

**Figure 4 Analysis of RNA and protein profiles for long-term glucose starvation**

We group RNA, and protein, time courses based on general qualitative behaviors. In response to glucose starvation RNA can be shut off, turned on, pulsed up, and pulsed down. A) To sort the profiles, a piecewise continuous curve is fit to our data. The parameter, t0, is the time at which we start to collect data at 3hrs into growth. The curve was fit using a differential evolution fitting algorithm that is gradient free and population based, allowing for a range of possible parameter sets that can explain our data given the experimental error. B-F) Four random examples of measured RNA time courses averaged across 3 biological replicates (green circles) with their standard deviations (green bars) along with the corresponding fits (blue). The blue bars represent the standard deviation of the range of fits that agree with our data. Both experimental time courses and fits are normalized by the average of the time course. G) Most of the RNA’s begin to change between 6-8 hrs, when the cells begin to enter stationary phase. This is demonstrated by the histogram of t1, the time to the first inflection point.

**Figure 5. Term enrichment and general behaviors.**

Fitting the mRNA and protein profiles allowed us to estimate the underlying dynamics and differential regulation of each gene sorting them into high confidence bins describing their behavior. Genes where put into bins based upon if they where up regulated, down regulated, temporarily up regulated, or temporarily down regulated. The mRNA, or proteins, in each bin where then tested for enrichment of GO terms. A) and B) shows the average of the mRNAs in a given enriched term for mRNAs that are down and up regulated respectively. C) and D) shows the average of the proteins in a given enriched term for proteins that are down and up regulated respectively. There was no significant enrichment for either proteins or mRNAs that where temporarily up or down regulated. As a complementary approach we took the average of all proteins in a given Kegg pathway annotation. E) and F) show the average of the proteins in the Kegg pathway that are changing significantly. All the other terms where flat.

**Figure 6. Flux ratio profiles in long-term glucose growth.**

Flux ratios where computed using fiat flux software from GC-MS derived 13C constraints. Only one independent flux ratio showed a significant change over the course of the experiment. Flux ratios for (A)SER from GLY, (B) OYR from MAL upper branch, (C) PEP through TK upper branch, (D) PEP through PPP upper branch, E) PEP from OAA, (F) OAA from PEP, (G) P5P from G6P lower branch, (H) E4P through TK, and (I) GLY through serine. Of the flux ratios that changed significantly (P5P from G6P lower branch) they showed a large correlation (~0.85) with the corresponding ratio of proteins that catalyze those reactions while the corresponding mRNA ratio shows a much lower correlation (~0.5) with the flux ratios.

**Figure 7. Lipid A and phospholipid profiles in long term glucose growth.**

Lipid A and phospholipids were extracted from all samples for analysis by negative ion MALDI-TOF and ESI-MS mass spectrometry, respectively, and the 6 hour and 2 week representative samples are shown in this figure. A) Activation of the acyl-transferase PagP, adds a C16 chain to lipid A on the 2-position primary acyl chain, resulting in a m/z of ~2035. B) Modification of phospholipids by cyclopropanation of one unsaturated double bond is catalyzed by CFA synthase. Transcripts of CFA synthase increase at late times (green) consistent with modification of PE where as the level of CFA protein stays relatively flat at late time points. C) As represented here by the 6 hr sample, lipid A from all samples collected between 3 and 48 hrs contained one major peak at ~1797 m/z corresponding to wild type, hexa-acylated lipid A. As illustrated on the right by the 2 week sample, the 1 and 2 week (168 and 336 hr) time points show the addition of the C16 chain to lipid A. D) Phosphatidylethanolamine (PE) is shown here as a representation of similar results obtained in phosphatidylglycerol (PG). The phospholipid profiles of the samples remained relatively consistent with wild type *E. coli* phospholipid profiles until hour 8, when a gradual increase in a peak ~702.5 m/z began and became the predominant species by 2 weeks. This mass corresponds to the cyclopropanation of one unsaturated double bond within a PE molecule containing acyl chains totaling 33 carbons distributed between the two acyl chains.