Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary information

Bacterial Metabolism in Immediate Response to Nutritional Perturbation with Temporal and Network View of Metabolites

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Supplementary Text

Chemicals

All solvents, metabolite standards, and other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water was obtained from a Milli-Q system (Millipore, Schwalbach, Germany).

Culture and induction of nutritional perturbation

E. coli strain JM109 was used for the direct metabolite analysis. Cultures were incubated in Luria-Bertani medium (4 h, 150 rpm, 37°C). Bacterial cells were collected by centrifugation (6,000 *g*, 5 min, 37°C) and resuspended in Hank's balanced salt solution (HBSS) containing 5 μM phenol red (OD_{600} = 2). The cell suspension was incubated in a water bath (37°C) with constant stirring. A pulse of glucose was added to give a final concentration of 5% (w/v), and cell samples were harvested from the suspension both before and after glucose addition.

Sampling

Matrix solution (6 mg/mL 9-aminoacridine in 80% methanol) was used to quench intracellular metabolism. Each sampling was performed by mixing 10 μL of suspension with 60 μL of the pre-cooled matrix solution (–40°C). The sampling interval was fixed at 10 s. For each time-course sample acquisition, 24 samples were taken prior to the nutritional perturbation induction and 72 post-induction, resulting in a sample set of 96 time points over 16 min.

Mass spectrometry

For time-course metabolite analysis, a time-of-flight type matrix-assisted laser/desorption ionization mass spectrometry (MALDI-MS) instrument (AXIMA Performance, Shimadzu, Japan) was used. 1 μL of the analyte was applied onto a ground-steel MALDI sample plate and air-dried to give a sample spot. The spots were irradiated at a laser power that gave satisfactory ion intensity, and all analyses were performed using the same laser power in the negative ionization mode. Mass spectra were obtained by MALDI-MS analysis where five laser shots were accumulated and 256 spectra were averaged per spot. The analysis time was less than 20 s/spot. Four spots were deposited from an individual sample and averaged for use in further data analyses. Mass spectra were calibrated internally using the internal standard and peaks

that appear consistently.

Raw data processing

Peak pick, normalization, peak alignment, and scaling were conducted using an in-house Perl script. The cut-off threshold was 30-fold of noise intensity and mass error tolerance was 200 ppm. Ultimately, 100–200 peaks were detected per spectrum. Peaks that appeared in the blank sample (HBSS + 9-aminoacridine) or that were detected fewer times than half of the number of acquired spectra were excluded from the following statistical analysis. Peak intensity in a spectrum was normalized to give a zero mean and unit variance throughout the time course. Missing values were designated as not available.

Parameter optimization for single correlation network

In constructing a temporal correlation network, the appropriate adjustment of parameters is important. Although the length of the detection probe should be as short as possible to evaluate a short-term correlation, the sample size itself influences the quality of the detected correlation. The threshold level of the correlation coefficients is also critical for the resulting correlation network.

Because the result was dependent on r_0 and k, these parameters were determined based on graph theory parameters (Scheme S1). Parameter k, referred to as probe length, was involved in a trade-off between the correlation detection power and the shortest detectable correlation span. The correlation profile matrix **B** was subjected to two kinds of evaluation, edge density measurement and modularity measurement. The edge density was represented simply as the ratio of the entry of 1 in **B**. In the modularity measurement, every possible pair of correlation profiles in **B** (***b***) was subjected to a pairwise comparison, giving a similarity score. The similarity score function was calculated as $s = u/v$, where u represented the minimum difference between time points when the correlation indicators of two metabolite pairs changed from negative to positive, and v represented the sum of time points where both indicators were positive, formulated as $\left(\boldsymbol{b}_1\right)^{\mathrm{T}} \boldsymbol{b}_2$. Although v could also be regarded as temporal similarity, this measure was vulnerable to chance coincidences. Therefore, s was regarded as the degree of simultaneity of the temporal metabolite correlations. Because s is produced for every experimental replication, replicated similarity values were represented by the minimum one, which represents the worst case of the simultaneity, which was regarded

as an 'honest' estimation. The resulting similarity score profile is a vector with a length of ${}_pC_2$, which can also be regarded as a peak list of the similarity network where nodes represent a pair of two correlation profiles and edges represent the temporal similarity of the two profiles. In the similarity network, communities were extracted by deleting nodes with the highest degree of betweenness at the corresponding iteration step to achieve the highest modularity. The weight threshold for peak selection was optimized to also maximize the modularity. The higher modularity was desirable to illustrate temporal trends of metabolite-metabolite correlations that would appear in specific timings. As the result, these two parameters were adjusted to give an ideal balance of the graph theory properties (graph density and modularity) of the resulting network (k = 16 and $r_0 = 0.85$, Figure S1).

Threshold adjustment for partial correlation profile

The sum of edges in t-th time window ($\boldsymbol{b}_t' \cdot \mathbf{1}$) is dependent on F_t. To achieve a consistent visualization of the correlation profile, F_t adjusted for each t. Initially, F_1 was set to give at least five edges in the correlation network. To illustrate clearly the time-dependent variance of partial correlation, F_t ($t \geq 2$) for the following time windows was determined depending on the previous correlation profile.

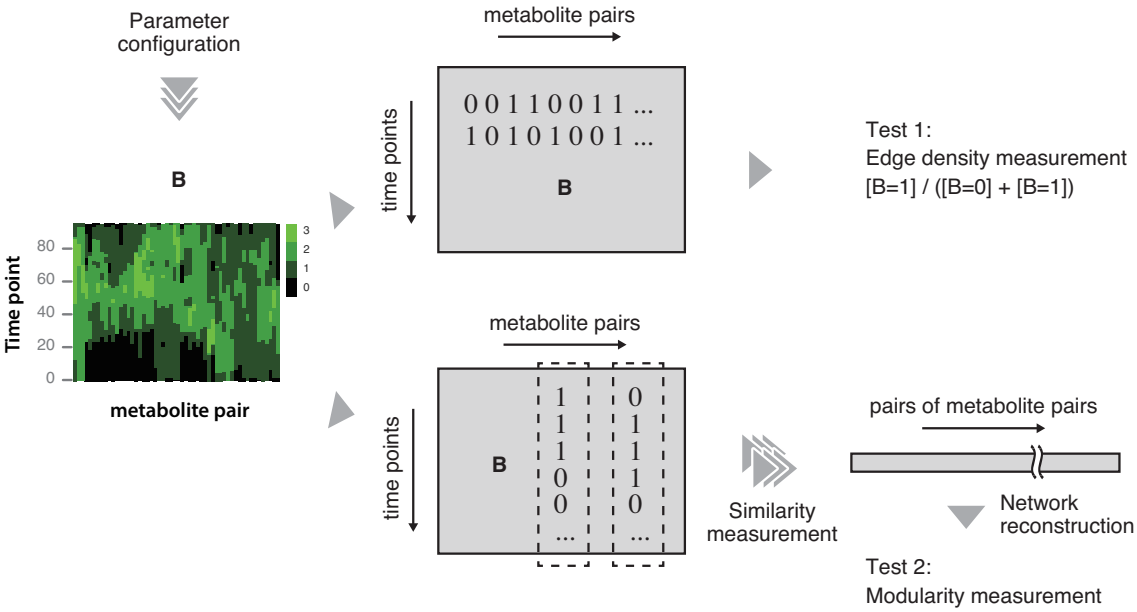
$$F_1 = \min\left\{x \middle| \boldsymbol{b}_1' \cdot \mathbf{1} \geq 5\right\}$$

$$F_t = \max\left\{x \middle| 0 \leq x \leq 0.4, S_t(x) \geq 0.8, n_{11} \geq 5\right\}$$

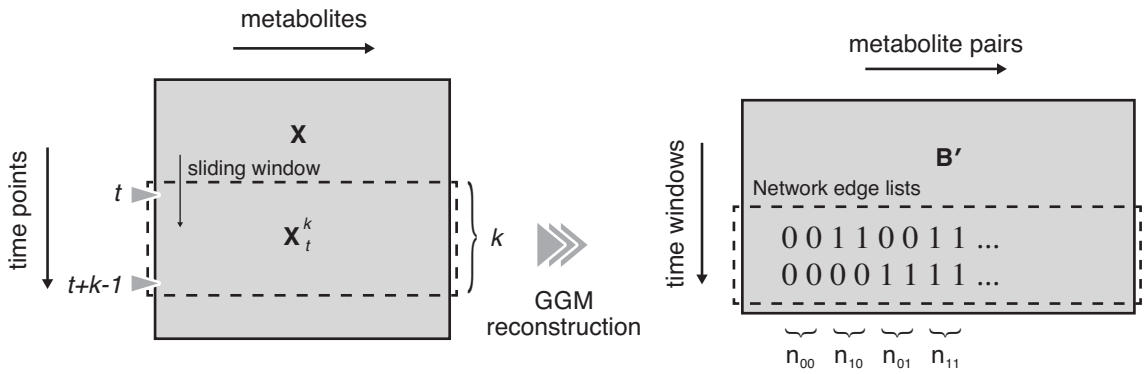
$$S_t = \frac{n_{11}}{n_{11} + n_{10} + n_{01}}$$

The similarity index S_t is the degree of identification with regard to the t-th and the previous time windows. The term n_{11} is the number of edges that are significant under a given FDR threshold, both in the t-th and the previous time window. The terms n_{10} and n_{00} indicate the number of edges of either or neither of the adjacent time windows, respectively. The threshold numbers in the formula were representative. The principle regulation was that FDR threshold was always less than 0.4, indicating the proportion of false-positive edges was 40% at most. Small variations in these constants led to no significant changes in the result. A series of $\boldsymbol{b}'$ was then reconstructed into a partial correlation indicator matrix $\mathbf{B}'$ with $T - k + 1$ rows and p columns.

Supplementary Figures



Scheme S1. Evaluation of parameters used in the single correlation analysis.



Scheme S2. Construction of temporal graphical Gaussian models (GGMs) using a sliding window.

The width of the sliding window was identical to that tuned in the single correlation analysis (Scheme 1). The partial correlation coefficients threshold (cutoff) was tuned for each pair of successive partial correlation profiles.

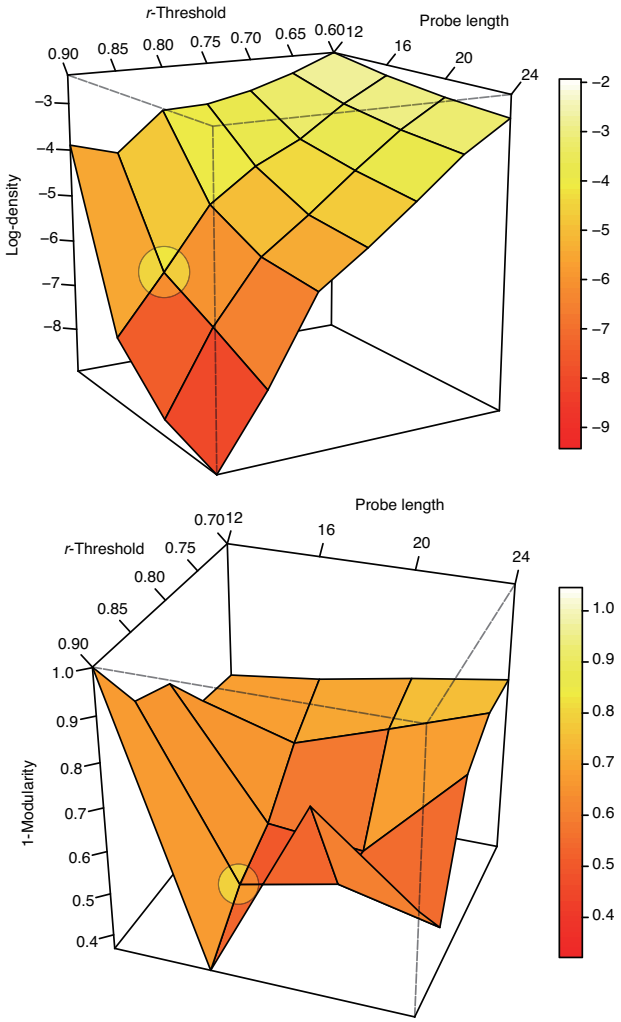


Figure S1. Relationship of parameters for correlation analysis with the properties of the resulting similarity network.

A. Density of the similarity network corresponding to a given correlation coefficient threshold and significance level. As the network density monotonically decreased with increases in the two parameters (*r*-threshold (r_0) and width of sliding window (k)), we selected parameter values so that a drop of density was observed ($r_0 = 0.85$, $k = 16$). **B.** Modularity on the similarity networks achieved under the same set of conditions as in **A**. Higher modularity was observed when the estimated optimum parameters were applied, supporting the validity of selected parameters.