Supplementary Information

<u>Title:</u> A U–system approach for predicting metabolic behaviors and responses based on an alleged metabolic reaction network

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Simple Michaelis-Menten system

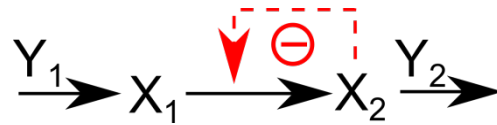A simple model of a linear pathway with inhibition is sketched in Figure S1.

Figure S1. **Linear pathway with inhibition (⊖).** X_1 and X_2 represent metabolites whereas Y_1 and Y_2 represent enzyme activities for the X_1 influx and X_2 efflux, respectively.

Flux expressions and parameters for this pathway were constructed in the typical Michaelis-Menten format where the enzymatic reaction velocity is usually determined by an *in vitro* experiment [6]. The usual Michaelis-Menten model with inhibition is presented in equations (S1) and (S2),

$$\frac{dX_1}{dt} = Y_1 - \frac{V_{max} X_1}{X_1 + K_m \left(1 + X_2 / K_i\right)} \tag{S1}$$

$$\frac{dX_2}{dt} = \frac{V_{max} X_1}{X_1 + K_m \left(1 + X_2 / K_i\right)} - Y_2 X_2 \tag{S2}$$

where V_{max}=100.0, K_m=10.0, K_i=5.0, Y_1=5.0, Y_2=2.0, and the steady state is $X_1^* = 0.789$, $X_2^* = 2.50$.

The corresponding GMA model with U–system simplifications is presented in equations (S3) and (S4),

$$\frac{dX_1}{dt} = Y_1 - X_1 X_2^{-0.5} \tag{S3}$$

$$\frac{dX_2}{dt} = X_1 X_2^{-0.5} - X_2 \qquad\qquad\qquad\qquad\qquad\qquad (S4)$$

where $Y_1 = 5.0$, $X_1^* = 11.180$, and $X_2^* = 5.0$

The dynamics of the time-varying metabolite concentrations was calculated for the Michaelis-Menten equations and the U–system approach of the GMA model. Specifically, the initial value of either X_1 or X_2 at $t{=}0$ was increased to twice the steady-state concentrations and the following trajectories were computed. Figure S2 shows the comparison of calculations from both models. It is clear that the results from both models are numerically quite different but qualitatively comparable. For both scenarios, when X_1 was perturbed, X_2 increased until it reached its maximum and decreased back to its steady-state, whereas X_1 continuously decreased. The results indicate that the qualitative shapes of the trajectories of metabolite concentrations strongly depend on the network structure, but less so on specific parameter values, as it was observed elsewhere [7]. Nonetheless, the metabolite concentrations at each time point and the time-scale of metabolite concentrations are quite different.
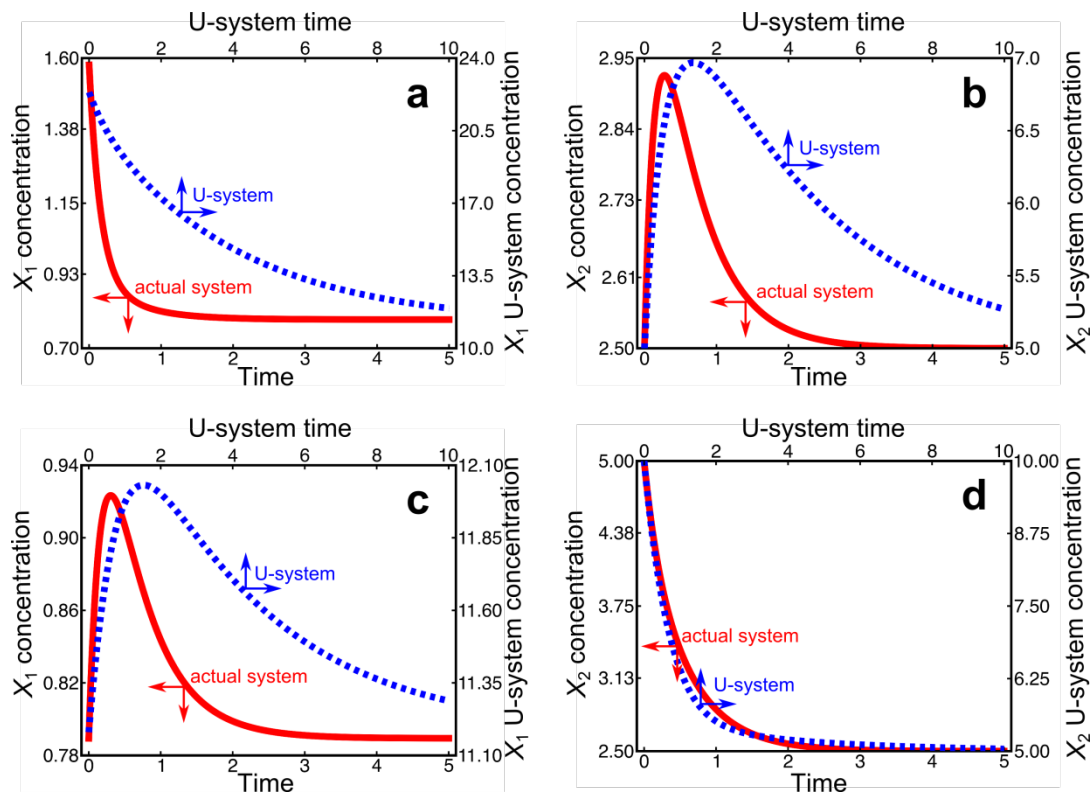
Figure S2. **Comparisons of time courses of X_1 and X_2 obtained by the Michaelis-Menten model and those by the simplified U–system approach of the corresponding GMA model.** The x-axis represents time, while the y-axis represents the concentrations of X_i. The red lines pertain to the actual system in left and bottom axes whereas the blue dotted lines are for U–system in right and top axes.

a   The X_1 U–system concentration compared with real concentration when the values of X_1 was increased two-fold at t=0.

b   The X_2 U–system concentration compared with real concentration when the values of X_1 was increased two-fold at t=0

c   The X_1 U–system concentration compared with real concentration when the values of X_2 was increased two-fold at t=0.

d   The X_2 U–system concentration compared with real concentration when the values of X_2 was increased two-fold at t=0.

Branched pathway

These supplements contain additional figures for the branched pathway model while the details for *Arabidopsis* model are provided in Usystem_SupplementaryModel.pdf with the U-system simulations comparing with experimental data from metabolome and amino acids analysis in Usystem_SupplementaryFigures.pdf, as mentioned in the body of the text.
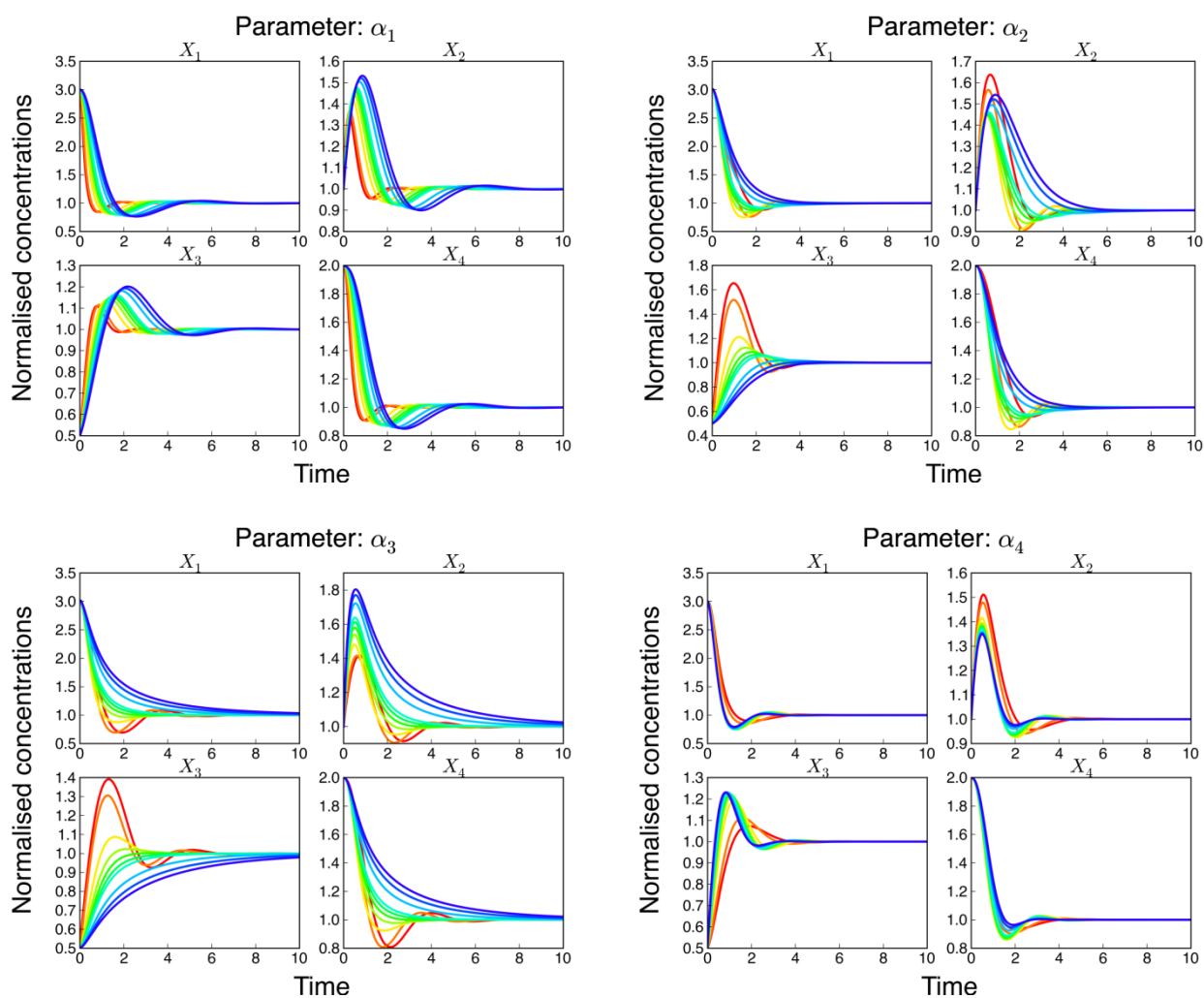

Figure S3. Normalized concentrations of X_i with variations of parameters α_i which value from 0.5 (red), 1, 5, 10, 15, 20, 25, 50, 75 to 100 (blue)
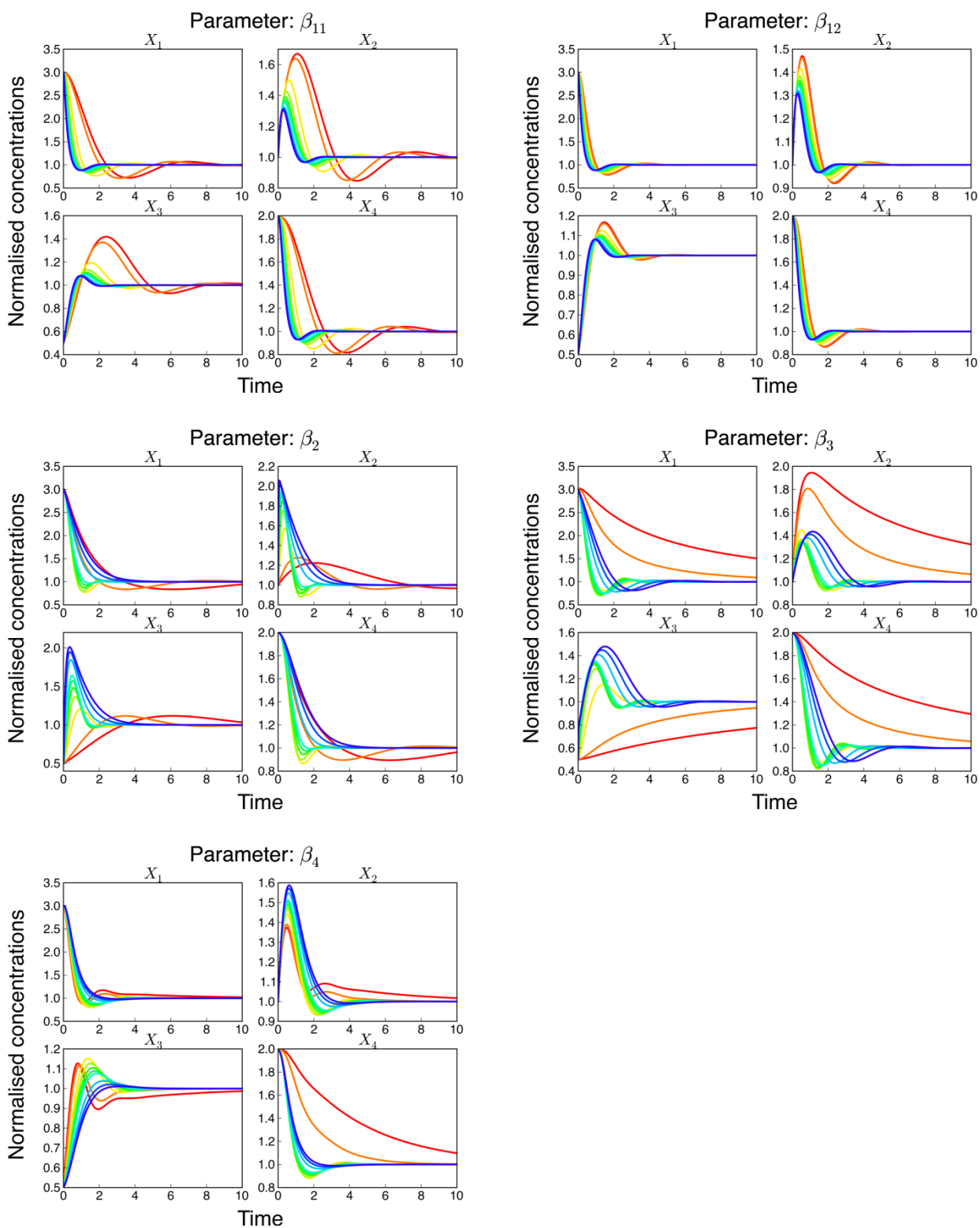
Figure S4. Normalized concentrations of X_i with variations of parameters β_i which value from 0.5 (red), 1, 5, 10, 15, 20, 25, 50, 75 to 100 (blue)
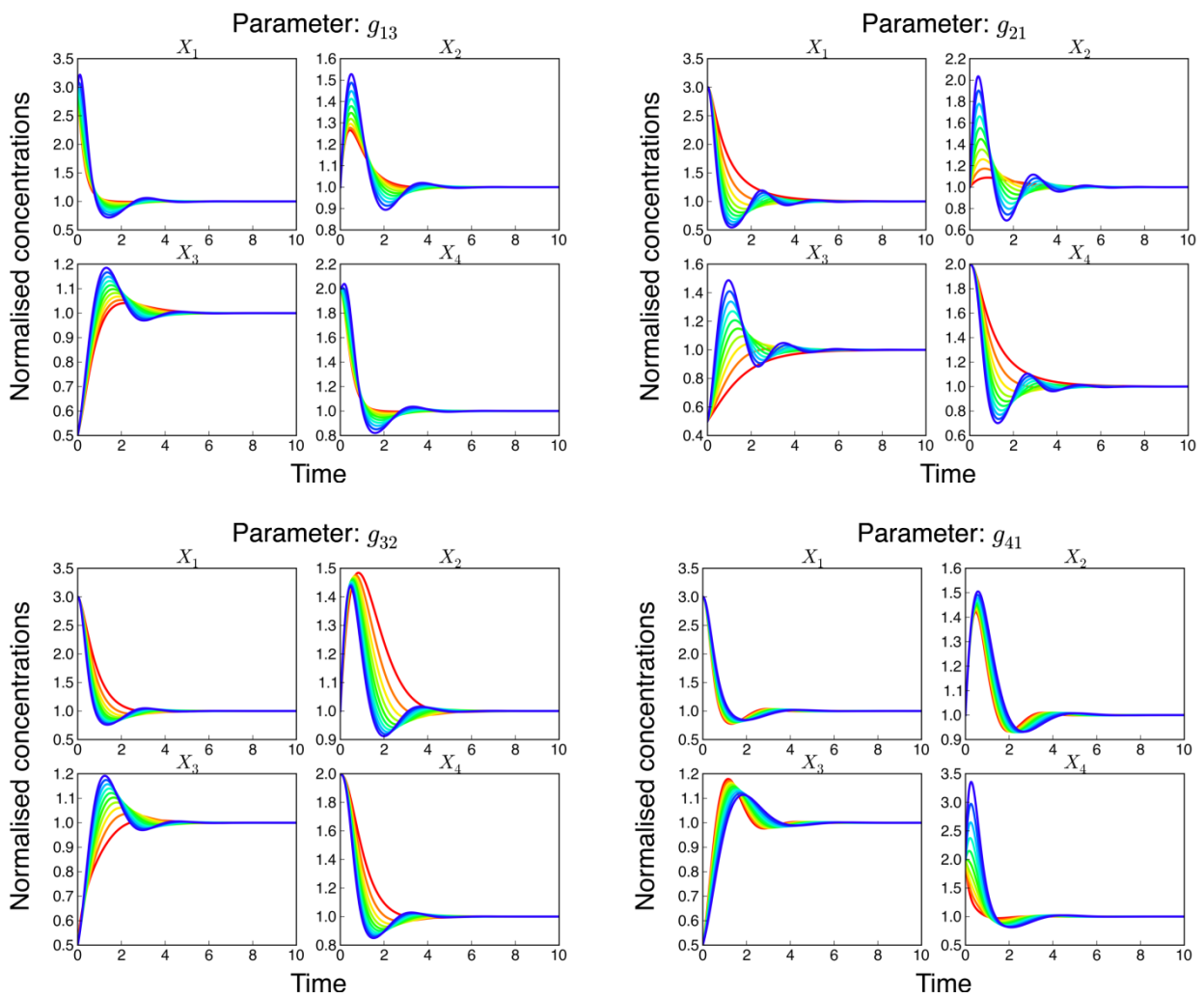
Figure S5. Normalized concentrations of X_i with variations of parameters g_{ij} which range from 0.1 (or –0.1; red) to 1.0 (or –1.0; blue)
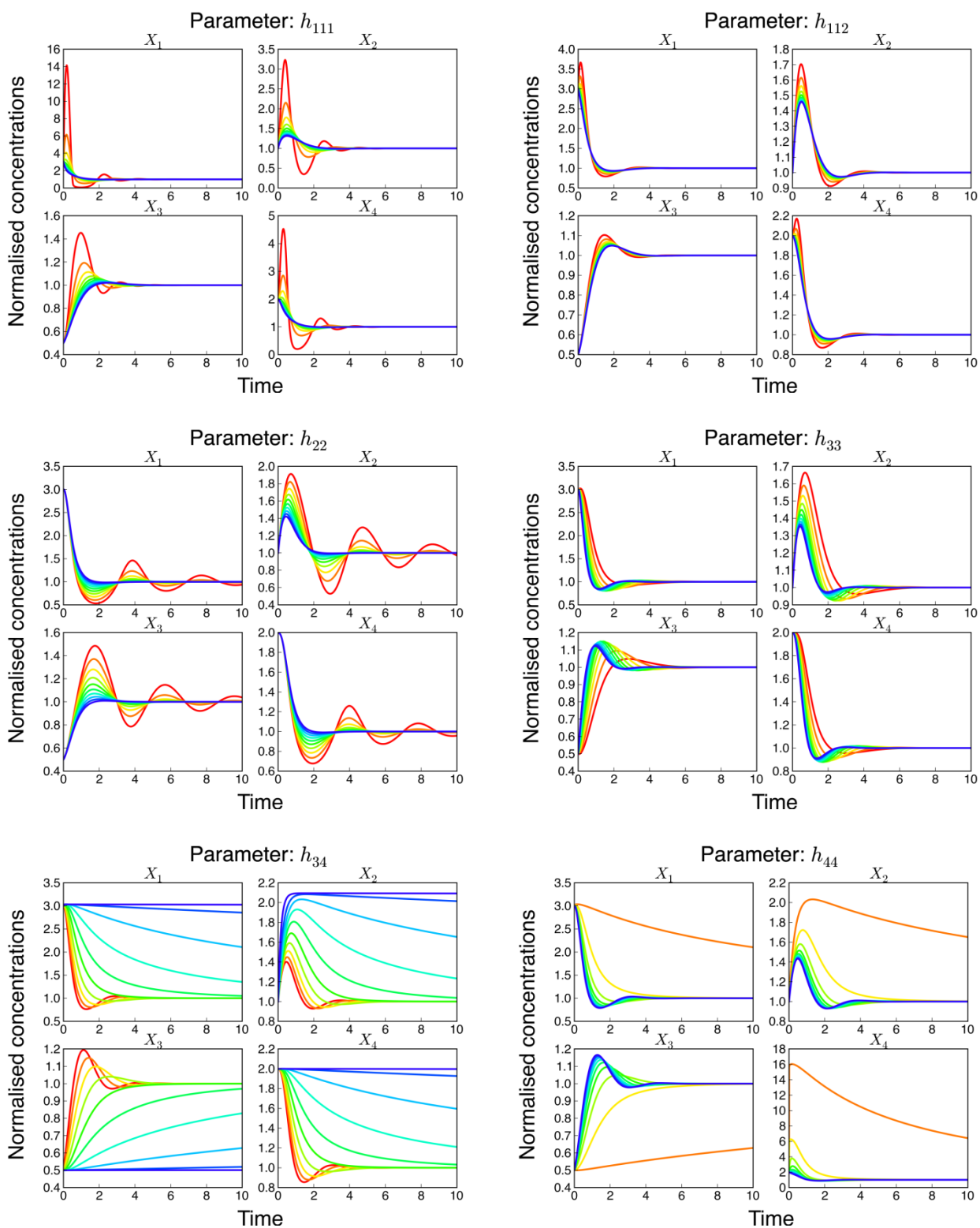
Figure S6. Normalized concentrations of X_i with variations of parameters h_{ij} which range from 0.1 (red) to 1.0 (blue)