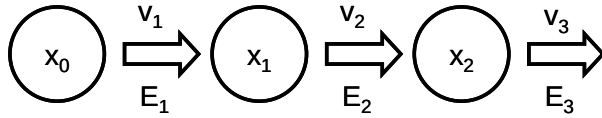


Supplementary Text 1 An example of the estimation of internal enzyme reaction rates.

An example of a small system is shown to explain how to calculate internal enzyme reaction rates from metabolite concentrations and reaction rates of system boundary enzymes. Consider a metabolic system consisting of three metabolites and three enzymes:



where x_0 , x_1 , and x_2 are metabolites; E_1 , E_2 , and E_3 are enzymes; and v_1 , v_2 , and v_3 are enzyme reaction rates. The mass balance of this system can be expressed by the following simultaneous ordinary differential equations:

$$\begin{aligned}\frac{dx_0}{dt} &= -v_1 \\ \frac{dx_1}{dt} &= v_1 - v_2 \\ \frac{dx_2}{dt} &= v_2 - v_3\end{aligned}$$

Accordingly, the overall system can be described by the following equation:

$$\begin{bmatrix} -1 & 0 & 0 & -1 & 0 & 0 \\ 1 & -1 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ \frac{dx_0}{dt} \\ \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \end{bmatrix} = \mathbf{0}$$

In this system, E3 is a system boundary enzyme. When the kinetics of E3 is known and all metabolite concentrations are measurable, this equation can be separated to an unknown-variables

(v_1 and v_2) part and a known-variables part:

$$\begin{bmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \end{bmatrix} + \begin{bmatrix} 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ -1 & 0 & 0 & -1 \end{bmatrix} \cdot \begin{bmatrix} v_3 \\ \frac{dx_0}{dt} \\ \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \end{bmatrix} = \mathbf{0}$$

Thus, the equation to estimate v_1 and v_2 can be written as follows:

$$\begin{bmatrix} v_1 \\ v_2 \end{bmatrix} = - \begin{bmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{bmatrix}^{\#} \cdot \begin{bmatrix} 0 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ -1 & 0 & 0 & -1 \end{bmatrix} \cdot \begin{bmatrix} v_3 \\ \frac{dx_0}{dt} \\ \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \end{bmatrix}$$

denotes the Moore-Penrose pseudo-inverse. By substituting values of v_3 , dx_0/dt , dx_1/dt , and dx_2/dt at each sampling point into the above equation, time series of v_1 and v_2 can be obtained.

Table S1 Initial metabolite concentrations.

The metabolite concentrations marked with asterisks were constant at the initial values.

A. E. coli

Metabolite	Concentration (mM)
Glc _x	5.56×10^{-2}
G6P	3.48
F6P	6.00×10^{-1}
FDP	2.72×10^{-1}
DHAP	1.67×10^{-1}
GAP	2.18×10^{-1}
PGP	8.00×10^{-3}
3PG	2.13
2PG	3.99×10^{-1}
PEP	2.67
Pyr	2.67
6PG	8.08×10^{-1}
Ribu5P	1.11×10^{-1}
Xyl5P	1.38×10^{-1}
Sed7P	2.76×10^{-1}
Rib5P	3.98×10^{-1}
E4P	9.80×10^{-2}
G1P	6.53×10^{-1}
AMP*	9.55×10^{-1}
ADP*	5.95×10^{-1}
ATP*	4.27
NADP*	1.95×10^{-1}
NADPH*	6.20×10^{-2}
NAD*	1.47
NADH*	1.00×10^{-1}

B. S. cerevisiae

Metabolite	Concentration (mM)
Glc _o [*]	2.50
Glc _x	3.33×10^{-2}
Glc	3.71×10^{-3}
G6P	5.71×10^{-1}
F6P	7.20×10^{-2}
FDP	5.09×10^{-2}
DHAP	2.85×10^{-1}
GAP	1.24×10^{-2}
PGP	3.13×10^{-5}
PEP	6.27×10^{-3}
Pyr	6.54×10^{-2}
ACA	1.27×10^{-1}
ACA _x	1.10×10^{-1}
EtOH	3.75
EtOH _x	3.21
Glyc	3.64×10^{-1}
Glyc _x	1.46×10^{-1}
CN _x	5.56
CN _o [*]	5.60
AMP	6.04×10^{-1}
ADP	1.76
ATP	1.57
NAD	7.79×10^{-1}
NADH	2.01×10^{-1}

Table S2 Smoothing functions applied to noisy metabolite concentration time series.

Name	Formula
poly1	$C(t) = p_1 \cdot t + p_2$
poly2	$C(t) = p_1 \cdot t^2 + p_2 \cdot t + p_3$
poly3	$C(t) = p_1 \cdot t^3 + p_2 \cdot t^2 + p_3 \cdot t + p_4$
poly4	$C(t) = p_1 \cdot t^4 + p_2 \cdot t^3 + p_3 \cdot t^2 + p_4 \cdot t + p_5$
poly5	$C(t) = p_1 \cdot t^5 + p_2 \cdot t^4 + p_3 \cdot t^3 + p_4 \cdot t^2 + p_5 \cdot t + p_6$
rat11	$C(t) = \frac{p_1 \cdot t + p_2}{t + p_3}$
rat22	$C(t) = \frac{p_1 \cdot t^2 + p_2 \cdot t + p_3}{t^2 + p_4 \cdot t + p_5}$
rat31	$C(t) = \frac{p_1 \cdot t^3 + p_2 \cdot t^2 + p_3 \cdot t + p_4}{t + p_5}$

$C(t)$, metabolite concentration time series (mM); t , time (s) ; p_n , n -th parameter of the time function.

Table S3 Parameters of smoothing functions applied to noisy metabolite concentration time series.

(A)

Metabolite	Glc _x	G6P	F6P	FDP	DHAP	GAP	PGP	3PG	2PG
Formula	poly1	rat22	rat22	rat22	rat22	rat22	rat31	rat22	rat31
p ₁	-2.0118 ×10 ⁻³	3.5917	6.1787× 10 ⁻¹	1.2581	3.8058× 10 ⁻¹	4.4714× 10 ⁻¹	-2.8616 ×10 ⁻⁵	1.0841	-2.3092 ×10 ⁻³
p ₂	1.6109	-1.4389 ×10	-9.1722 ×10 ⁻²	-4.8399 ×10 ⁻¹	-6.3025 ×10 ⁻¹	-3.1038 ×10 ⁻¹	1.0654× 10 ⁻³	-8.3869	8.7585× 10 ⁻²
p ₃		2.3019× 10	3.0122× 10 ⁻¹	4.1135	1.7377	9.3203× 10 ⁻¹	-9.1496 ×10 ⁻³	3.4353× 10	-8.6563 ×10 ⁻¹
p ₄		-4.0590	-3.9900 ×10 ⁻¹	-4.1372	-3.2714	-1.4055	6.3836× 10 ⁻²	-3.2100	4.8161
p ₅		6.0083	5.3084× 10 ⁻¹	1.3659× 10	9.1551	4.1901	7.0708	1.7223× 10	1.0895× 10

(B)

Metabolite	PEP	Pyr	6PG	Ribu5P	Xyl5P	Sed7P	Rib5P	E4P	G1P
Formula	rat22	rat11	poly2	rat22	rat22	rat11	rat11	rat11	poly2
p ₁	1.3117	4.2778	-1.0415 ×10 ⁻³	1.3188× 10 ⁻¹	1.7556× 10 ⁻¹	1.9147× 10 ⁻¹	5.1540× 10 ⁻¹	1.6910× 10 ⁻¹	-1.8735 ×10 ⁻⁴
p ₂	-5.6198	1.4626	2.1053× 10 ⁻²	-5.2550 ×10 ⁻¹	-1.0527 ×10 ⁻¹	1.3057	2.3579× 10 ⁻¹	8.3945× 10 ⁻²	-5.9819 ×10 ⁻⁴
p ₃	6.3349× 10	5.7016× 10 ⁻¹	7.8927× 10 ⁻¹	6.2064× 10 ⁻¹	1.2366× 10 ⁻¹	4.9895	6.4828× 10 ⁻¹	1.0058	6.5185× 10 ⁻¹
p ₄	4.3019			-4.2332	-8.4976 ×10 ⁻¹				
p ₅	2.2247× 10			5.1292	8.9735× 10 ⁻¹				

Table S3 (Continued.)

(C)

Metabolite	Glcx	Glc	G6P	F6P	FDP	DHAP	GAP	PGP	PEP
Formula	rat11	poly5	poly2	poly2	poly5	poly4	poly4	poly3	poly4
P ₁	7.9886× 10 ⁻²	4.7503× 10 ⁻¹⁰	-2.9131 ×10 ⁻⁴	-3.2491 ×10 ⁻⁵	-1.0649 ×10 ⁻⁸	-6.2214 ×10 ⁻⁷	-3.5786 ×10 ⁻⁸	5.2856× 10 ⁻¹⁰	-9.2207 ×10 ⁻⁹
P ₂	1.5901× 10 ⁻²	-8.5157 ×10 ⁻⁸	2.6422× 10 ⁻²	3.0782× 10 ⁻³	1.1537× 10 ⁻⁶	9.2409× 10 ⁻⁵	5.1155× 10 ⁻⁶	-7.3373 ×10 ⁻⁸	1.3130× 10 ⁻⁶
P ₃	4.1942× 10 ⁻¹	5.5718× 10 ⁻⁶	5.8214× 10 ⁻¹	7.4694× 10 ⁻²	-1.9499 ×10 ⁻⁵	-4.6791 ×10 ⁻³	-2.4786 ×10 ⁻⁴	3.2859× 10 ⁻⁶	-6.3740 ×10 ⁻⁵
P ₄		-1.5717 ×10 ⁻⁴			-1.3878 ×10 ⁻³	8.8994× 10 ⁻²	4.4396× 10 ⁻³	2.5254× 10 ⁻⁵	1.1917× 10 ⁻³
P ₅		1.6347× 10 ⁻³			4.4834× 10 ⁻²	1.7593× 10 ⁻¹	7.3898× 10 ⁻³		4.9841× 10 ⁻³
P ₆									

(D)

Metabolite	Pyr	ACA	ACA _x	EtOH	EtOH _x	Glyc	Glyc _x	CN _x	ATP
Formula	poly4	poly3	poly3	poly4	poly2	poly4	poly4	poly2	rat22
P ₁	-7.2655 ×10 ⁻⁸	1.9992× 10 ⁻⁶	1.6963× 10 ⁻⁶	4.4071× 10 ⁻⁷	-2.7646 ×10 ⁻⁴	7.0661× 10 ⁻⁸	5.3770× 10 ⁻⁸	-1.0099 ×10 ⁻⁴	1.7557
P ₂	1.1154× 10 ⁻⁵	-2.4707 ×10 ⁻⁴	-2.0826 ×10 ⁻⁴	-5.4620 ×10 ⁻⁵	4.6656× 10 ⁻²	-9.4961 ×10 ⁻⁶	-7.8714 ×10 ⁻⁶	3.5016× 10 ⁻³	-5.3278 ×10
P ₃	-5.9903 ×10 ⁻⁴	9.7003× 10 ⁻³	8.4974× 10 ⁻³	1.6849× 10 ⁻³	3.0583	2.8632× 10 ⁻⁴	3.7106× 10 ⁻⁴	5.6207	5.0301× 10 ²
P ₄	1.2867× 10 ⁻²	1.2358× 10 ⁻¹	9.4787× 10 ⁻²	3.4301× 10 ⁻²		7.1687× 10 ⁻³	-3.6804 ×10 ⁻³		-3.2783 ×10
P ₅	5.6075× 10 ⁻²			3.6968		3.5349× 10 ⁻¹	1.5741× 10 ⁻¹		3.3690× 10 ²
P ₆									

Table S3 (Continued.)

(E)

Metabolite	ADP	AMP	NAD	NADH
Formula	poly5	poly5	rat22	poly5
P ₁	1.3700× 10 ⁻⁸	2.7583× 10 ⁻⁸	8.1936× 10 ⁻¹	3.2000× 10 ⁻⁹
P ₂	-2.8300 ×10 ⁻⁶	-4.9102 ×10 ⁻⁶	-1.9233 ×10	-7.6100 ×10 ⁻⁷
P ₃	2.0570× 10 ⁻⁴	3.1757× 10 ⁻⁴	2.1257× 10 ²	6.6000× 10 ⁻⁵
P ₄	-6.1334 ×10 ⁻³	-8.7275 ×10 ⁻³	-2.2396 ×10	-2.5037 ×10 ⁻³
P ₅	5.7833× 10 ⁻²	8.1022× 10 ⁻²	2.8200× 10 ²	3.6732× 10 ⁻²
P ₆				

(A) and (B), *E. coli*; (C), (D), and (E), *S. cerevisiae*. Formulas are shown in Table S2.

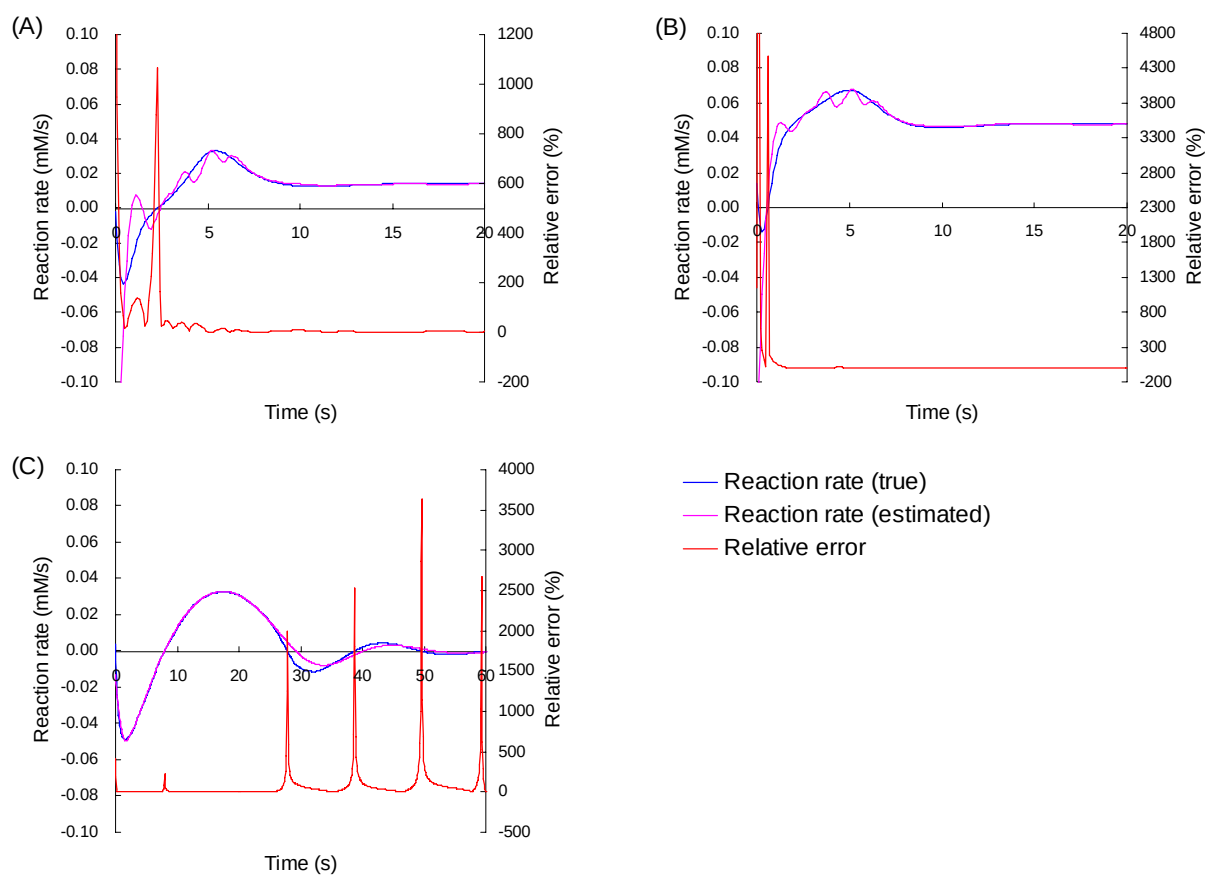


Figure S1 Relative error between true and estimated values of the enzyme reaction rate.

(A) *E. coli*, TKb; (B) *E. coli*, TA; (C) *S. cerevisiae*, AK.

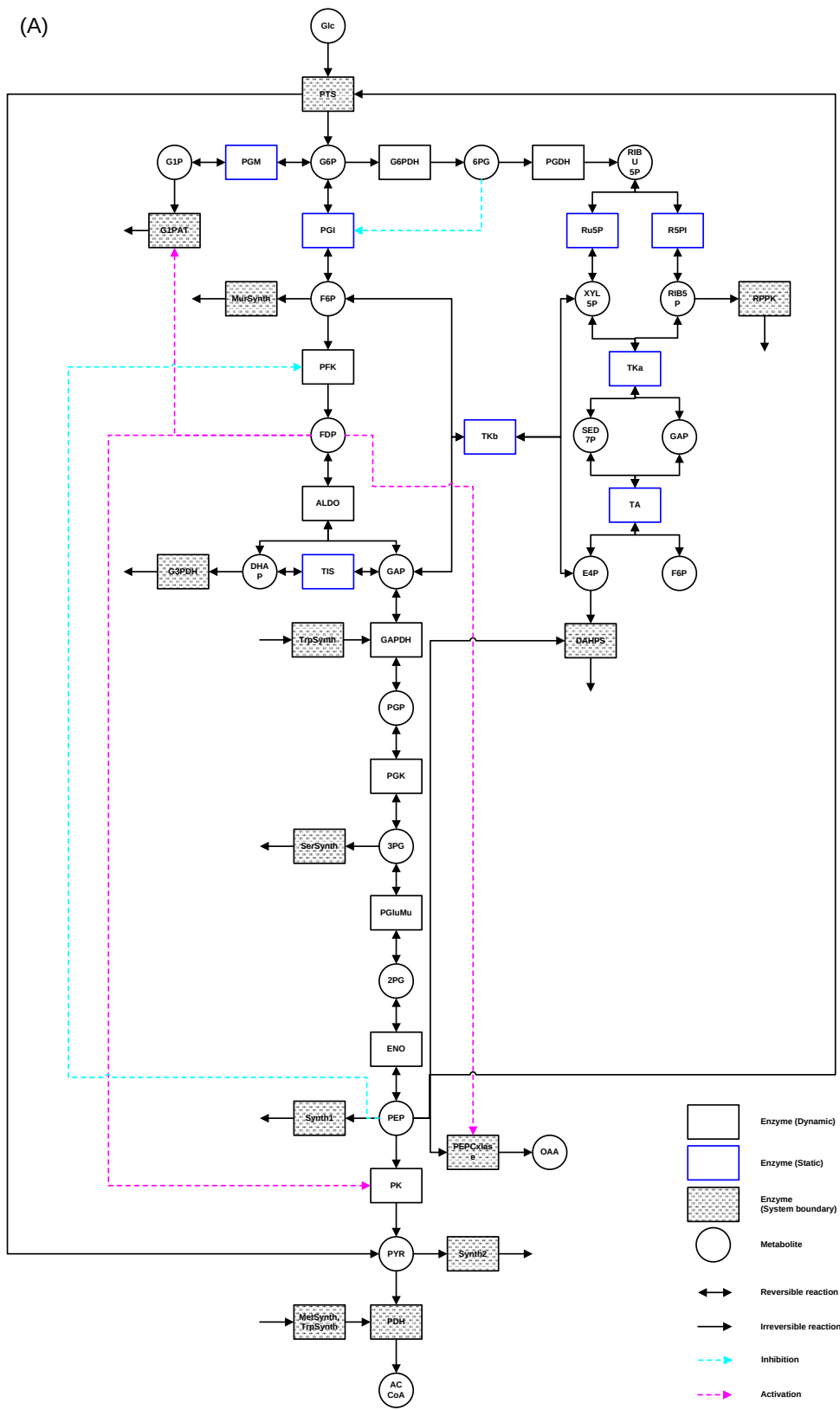


Figure S2

(B)

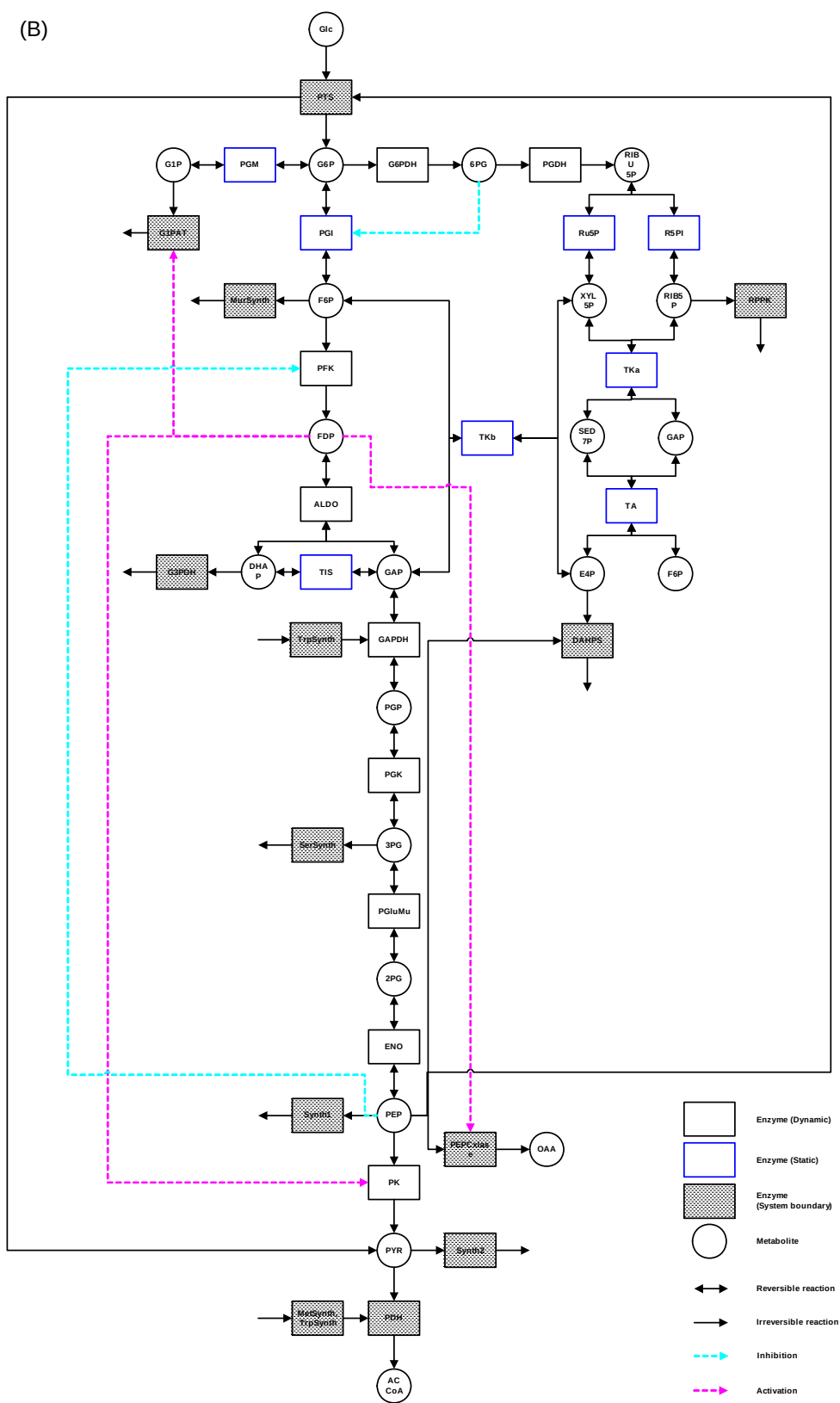


Figure S2 (Continued.)

(C)

Metabolic map of glycolysis and gluconeogenesis in *E. coli*. The map illustrates the flow of metabolites and the activity of enzymes, categorized by their dynamic behavior and regulatory interactions.

Legend:

- Enzyme (Dynamic): White box
- Enzyme (Static): Blue box
- Enzyme (System boundary): Grey box with diagonal lines
- Metabolite: Circle
- Reversible reaction: Double-headed arrow
- Irreversible reaction: Single-headed arrow
- Inhibition: Dashed cyan line
- Activation: Dashed magenta line

Metabolites (Circles): Glc, G1P, G6P, 6PG, RIB U 5P, Ru5P, R5P, XYL 5P, RIB 5 P, SED 7P, GAP, E4P, F6P, DHA P, PGP, 3PG, 2PG, PEP, PK, PYR, PDH, AC CoA, OAA.

Enzymes (Boxes):

- Dynamic (White):** PTS, PGM, G6PDH, PGD, PGI, F6P, PFK, ALDO, TIS, GAPDH, PGK, PGLuMu, ENO, PK, PDH.
- Static (Blue):** PGM, PGI, TKa, TKb, TA.
- System Boundary (Grey with diagonal lines):** G1PAT, MutS, TrpSynth, SerSynth, Synth1, Synth2, MeisSynth, TrpSynth, PEPck, PPK, DAPHS.

Regulatory Interactions (Dashed Lines):

- Inhibition (Dashed cyan):** 6PG inhibits PGI; F6P inhibits PFK; GAP inhibits TIS; PEP inhibits ENO; PEP inhibits PK.
- Activation (Dashed magenta):** G1P activates G1PAT; F6P activates MutS; F6P activates PFK; F6P activates TIS; F6P activates GAPDH; F6P activates PK; PEP activates PEPck; PEP activates PK.

Figure S2 (Continued.)

[illegible]

Figure S2 (Continued.)

(E)

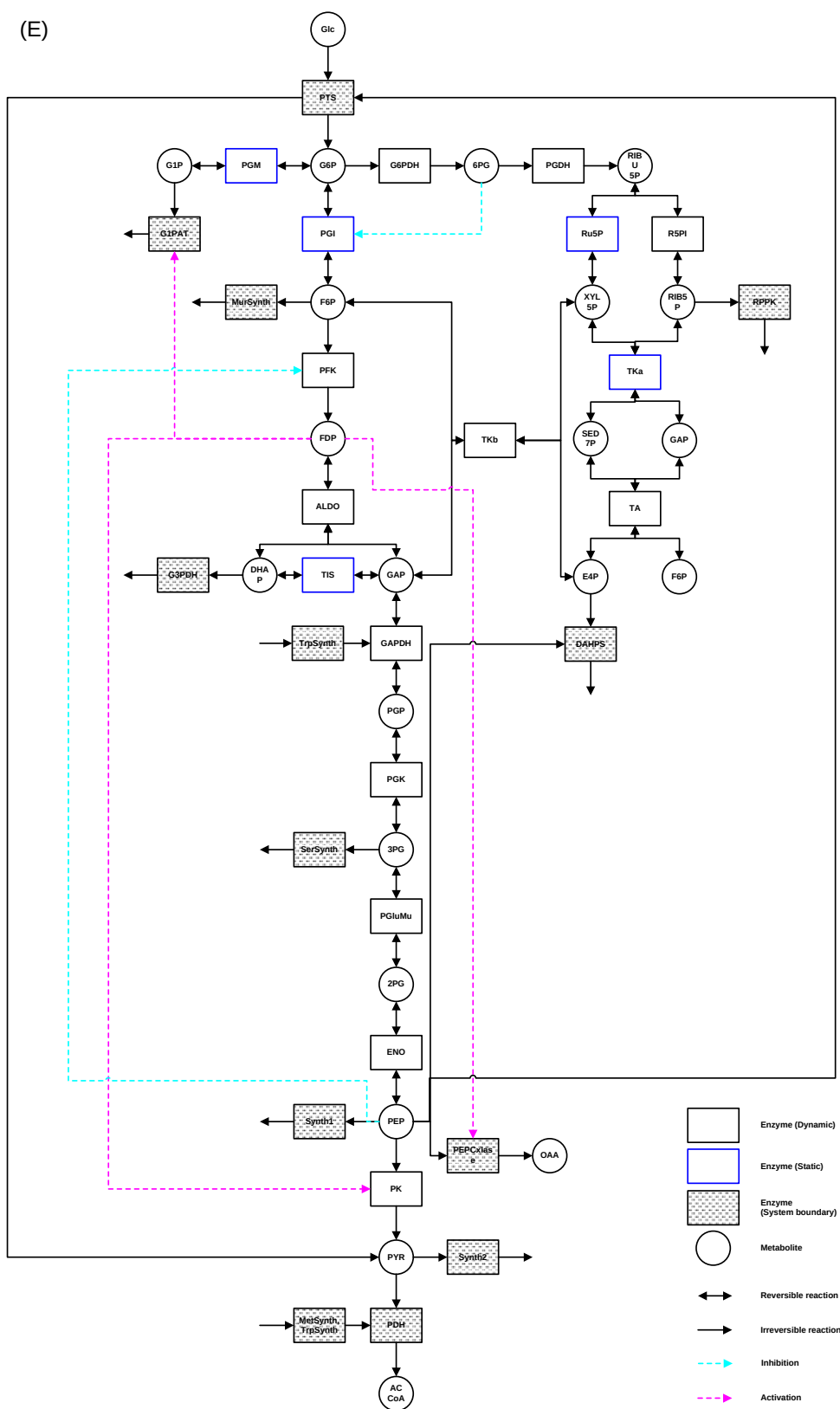


Figure S2 (Continued.)

(F)

Metabolic map of glycolysis and gluconeogenesis in *E. coli*. The map illustrates the flow of metabolites (circles) and the activity of enzymes (rectangles). Enzymes are categorized by their dynamic behavior: Dynamic (white), Static (blue outline), and System boundary (hatched). Metabolites are represented by circles. Reversible reactions are indicated by double-headed arrows, and irreversible reactions by single-headed arrows. Inhibition is shown with dashed cyan lines, and activation with dashed magenta lines.

Legend:

- Enzyme (Dynamic): White rectangle
- Enzyme (Static): Blue outline rectangle
- Enzyme (System boundary): Hatched rectangle
- Metabolite: Circle
- Reversible reaction: Double-headed arrow
- Irreversible reaction: Single-headed arrow
- Inhibition: Dashed cyan line
- Activation: Dashed magenta line

Figure S2 (Continued.)

(G)

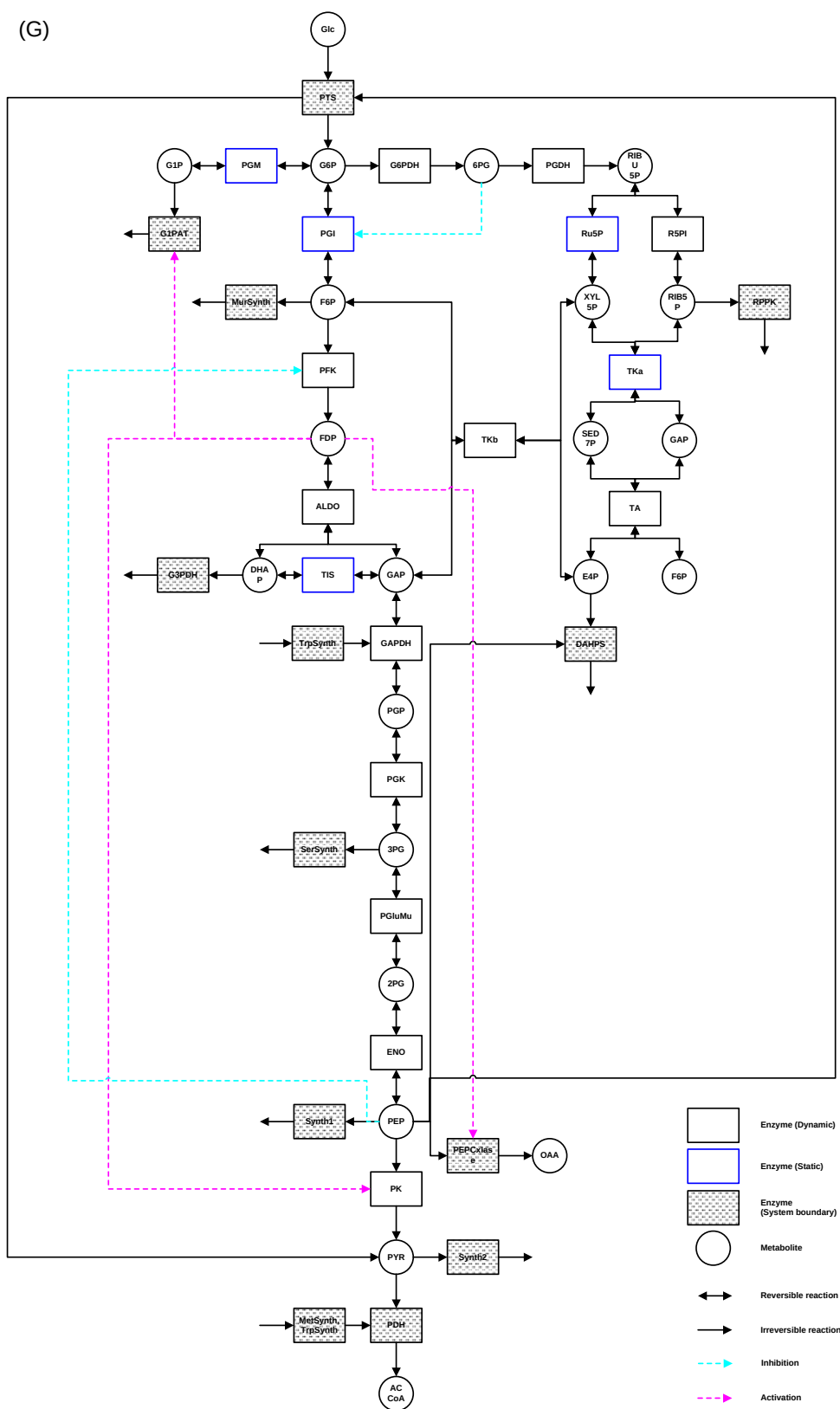


Figure S2 (Continued.)

(H)

Metabolic map of glycolysis and gluconeogenesis in *E. coli*. The map illustrates the flow of metabolites and the activity of enzymes, categorized by their dynamic behavior and system boundary.

Legend:

- Enzyme (Dynamic): White box
- Enzyme (Static): Blue box
- Enzyme (System boundary): Grey box with diagonal lines
- Metabolite: Circle
- Reversible reaction: Double-headed arrow
- Irreversible reaction: Single-headed arrow
- Inhibition: Dashed cyan line
- Activation: Dashed magenta line

Metabolites (Circles): Glc, G1P, G6P, 6PG, RIB U 5P, Ru5P, R5P, XYL 5P, RIB5 P, SED 7P, GAP, F6P, E4P, DAPHS, PEP, PYR, AC CoA, OAA.

Enzymes (Boxes):

- Dynamic (White):** PTS, PGM, G6PDH, PGD, PGI, F6P, PFK, ALDO, TIS, GAPDH, PGP, PGK, PGLuMu, 2PG, ENO, PK, PDH.
- Static (Blue):** PGM, PGI, TIS, TKa.
- System Boundary (Grey with diagonal lines):** G1PAT, MutS, TrpSynth, SerSynth, Synth1, Synth2, MeisSynth, TrpSynth, PEPCK, PPK, GAPDH, DHA P, DAPHS, OAA.

Key Features:

- Glycolysis Pathway:** Glc → G1P → G6P → 6PG → RIB U 5P → Ru5P → R5P → XYL 5P → RIB5 P → SED 7P → GAP → F6P → PFK → ALDO → TIS → GAPDH → PGP → PGK → PGLuMu → 2PG → ENO → PEP → PYR → PDH → AC CoA.
- Gluconeogenesis Pathway:** AC CoA → PDH → PYR → PEP → ENO → 2PG → PGLuMu → 3PG → PGK → PGP → GAPDH → TIS → ALDO → FDP → PFK → F6P → PGI → G6P → G1P → G1PAT.
- Regulation:** Inhibition (dashed cyan lines) and Activation (dashed magenta lines) are shown for various enzymes and metabolites.

Figure S2 (Continued.)

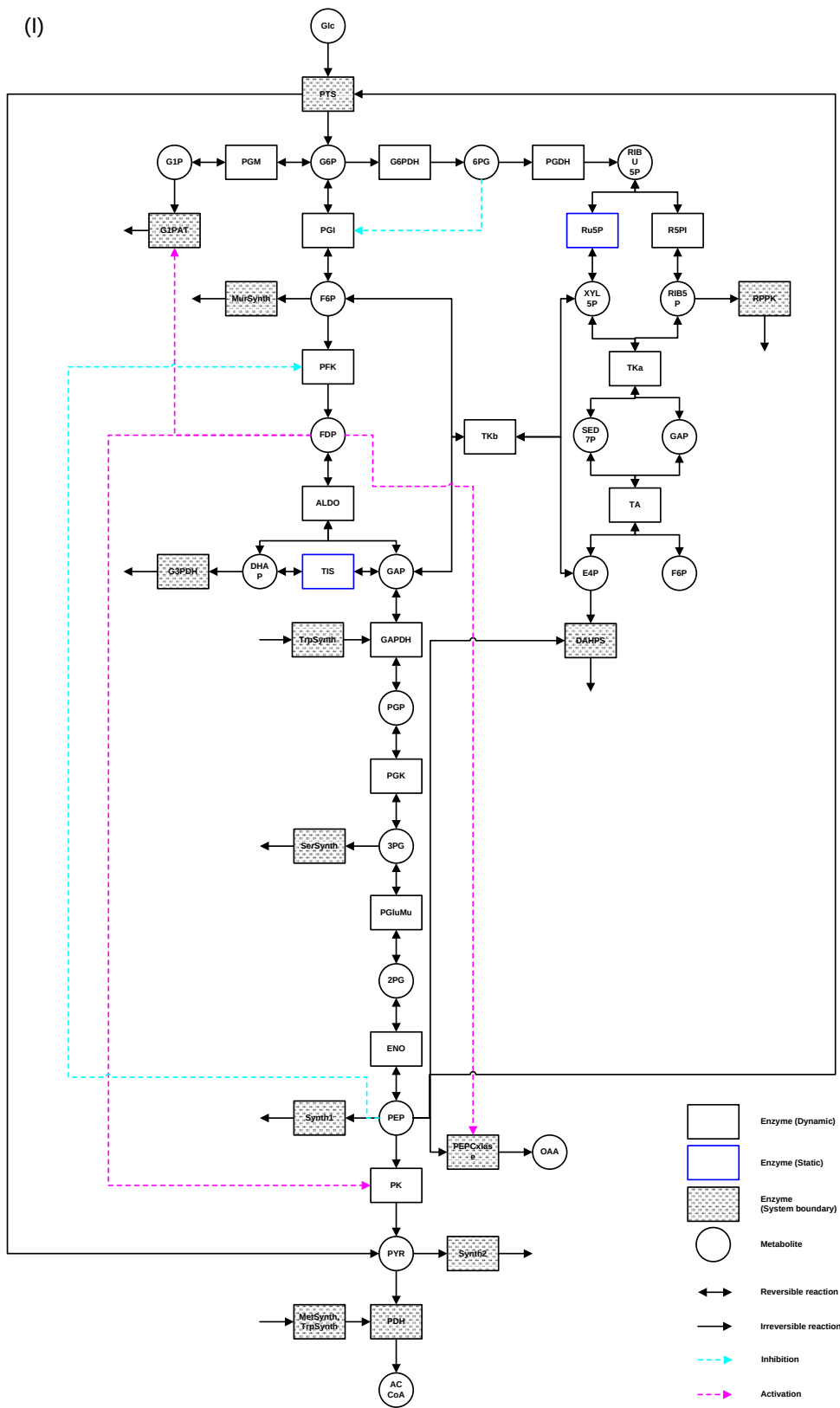


Figure S2 (Continued.)

(J)

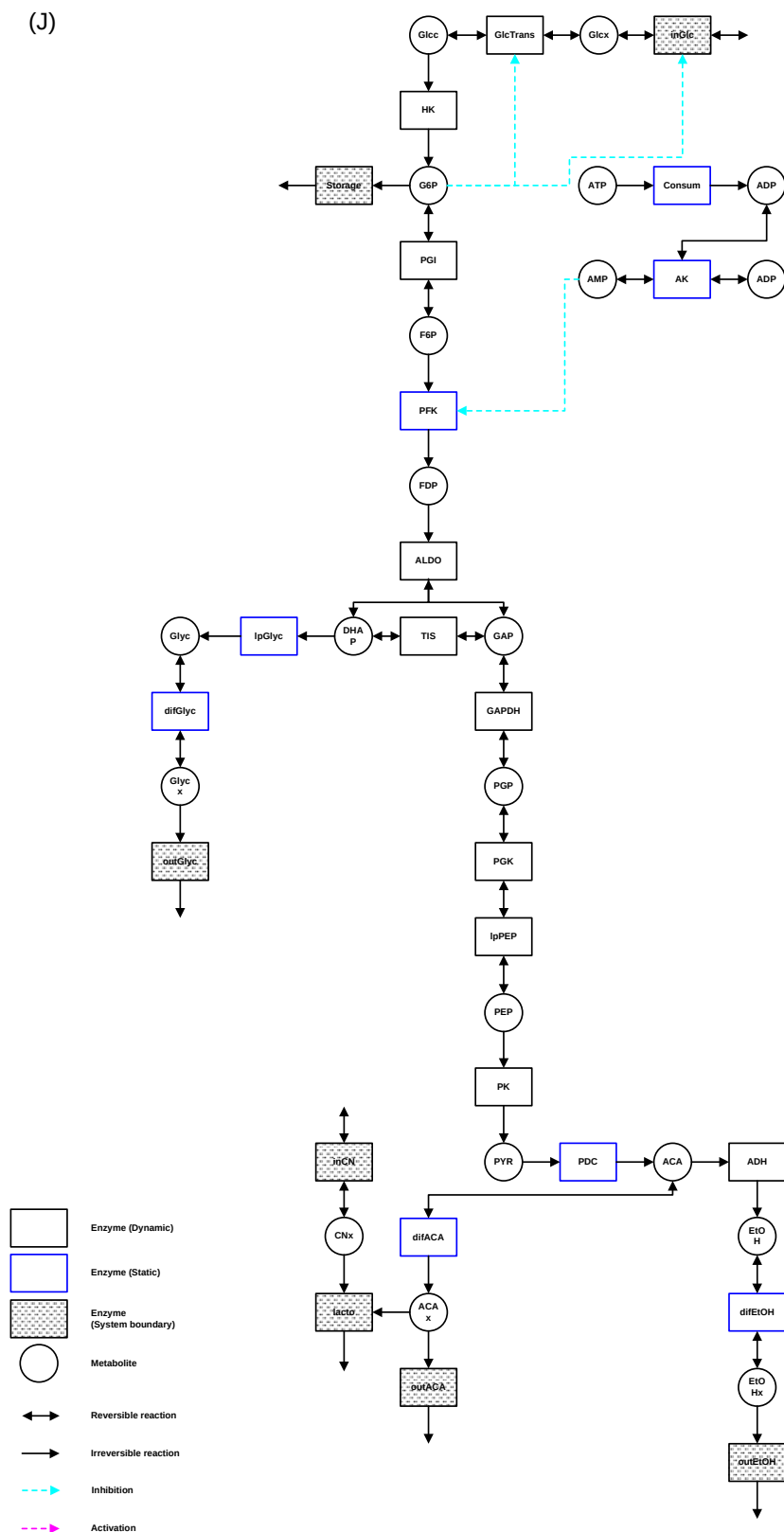


Figure S2 (Continued.)

(K)

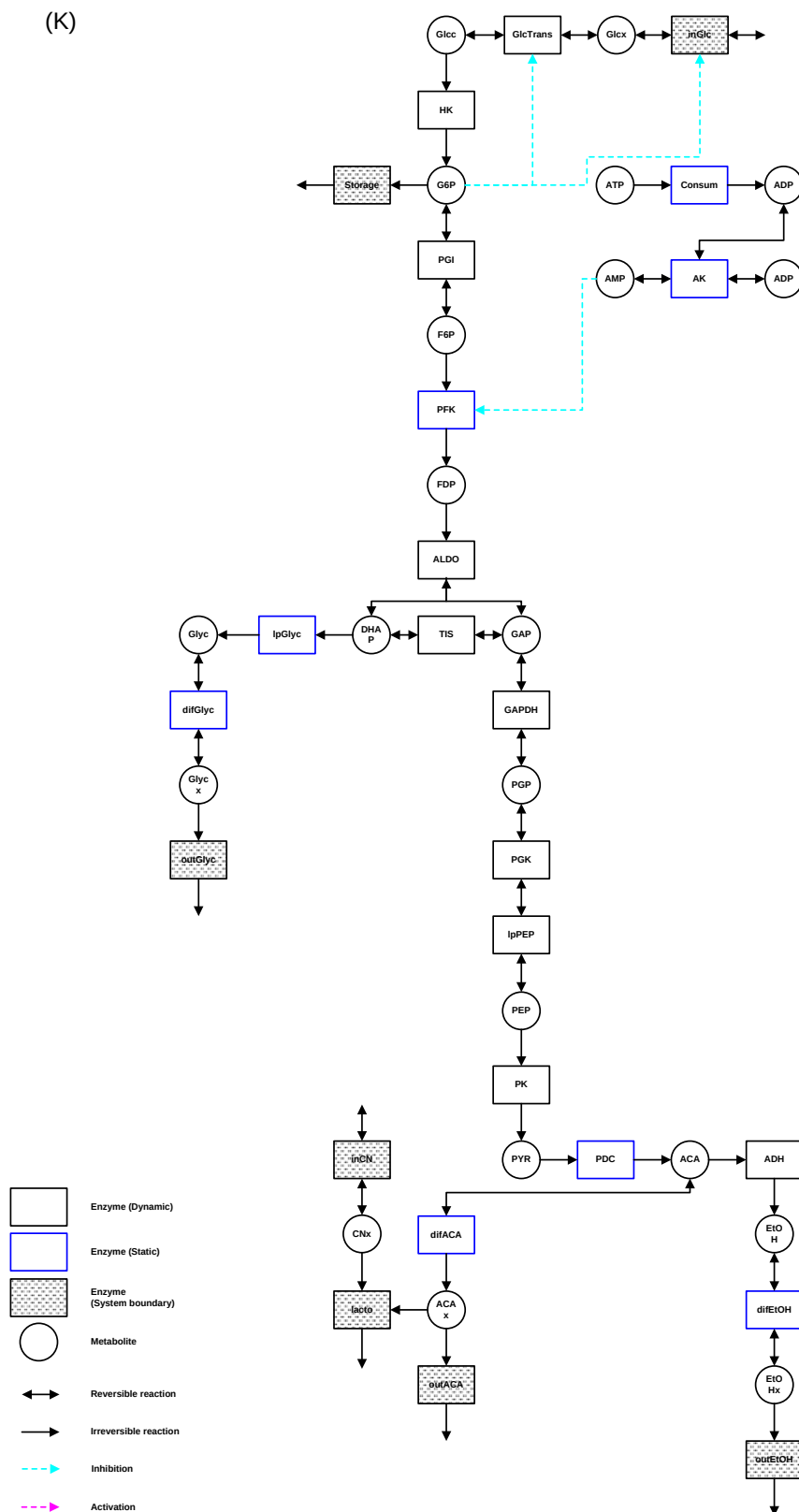


Figure S2 (Continued.)

(L)

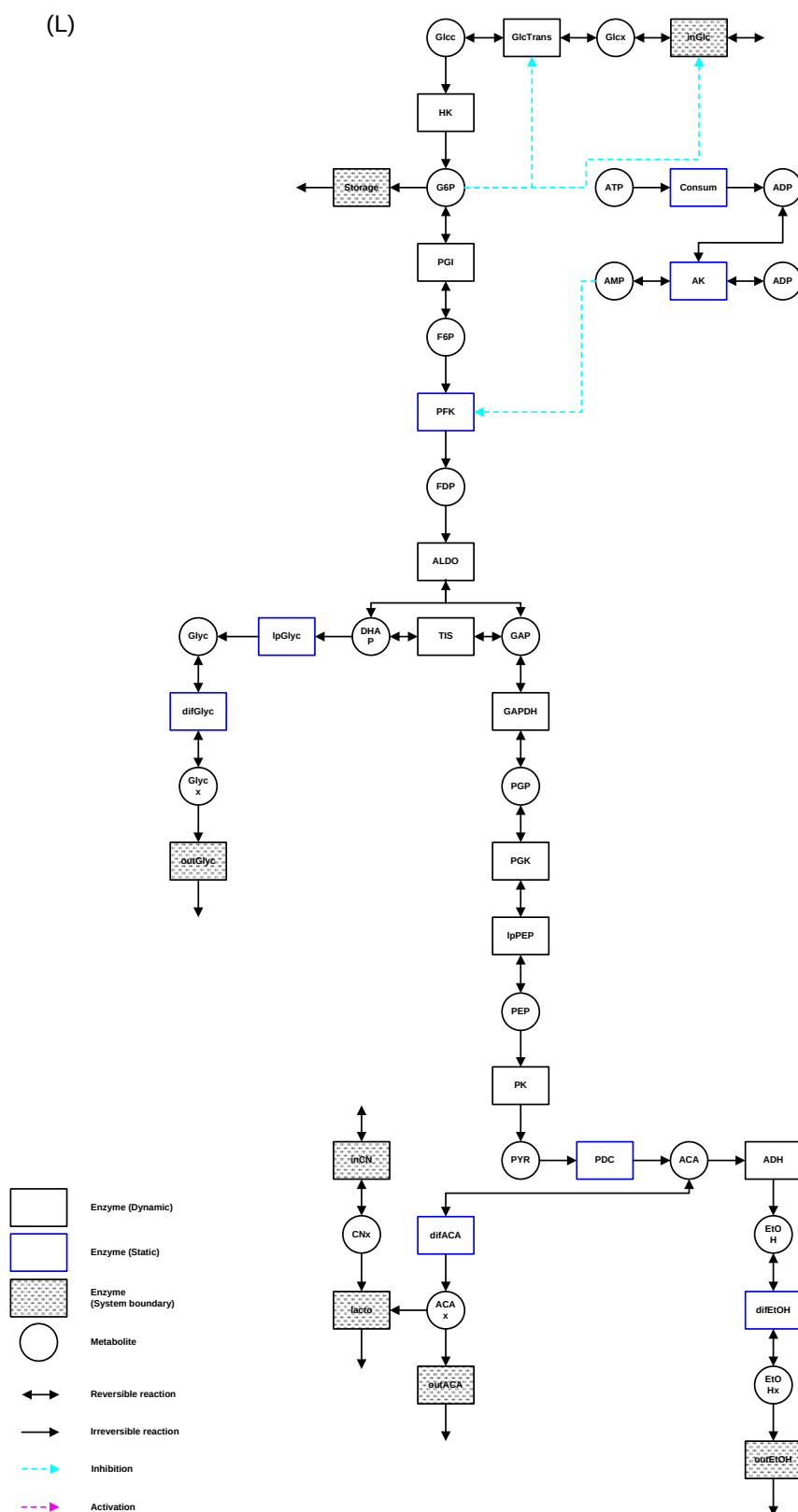


Figure S2 (Continued.)

(M)

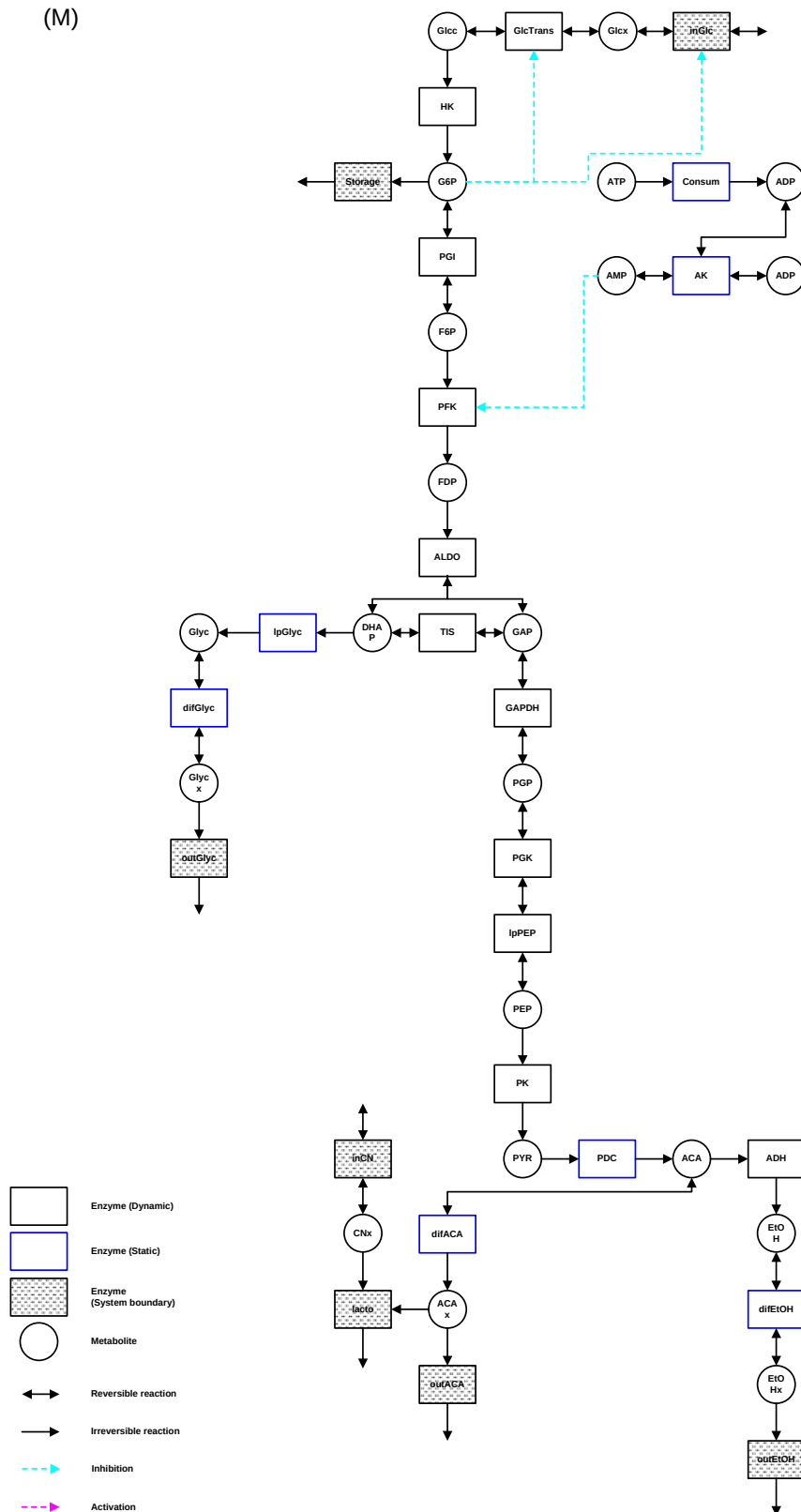


Figure S2 (Continued.)

(N)

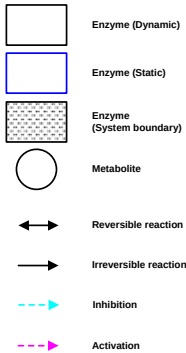


Figure S2 (Continued.)

(O)

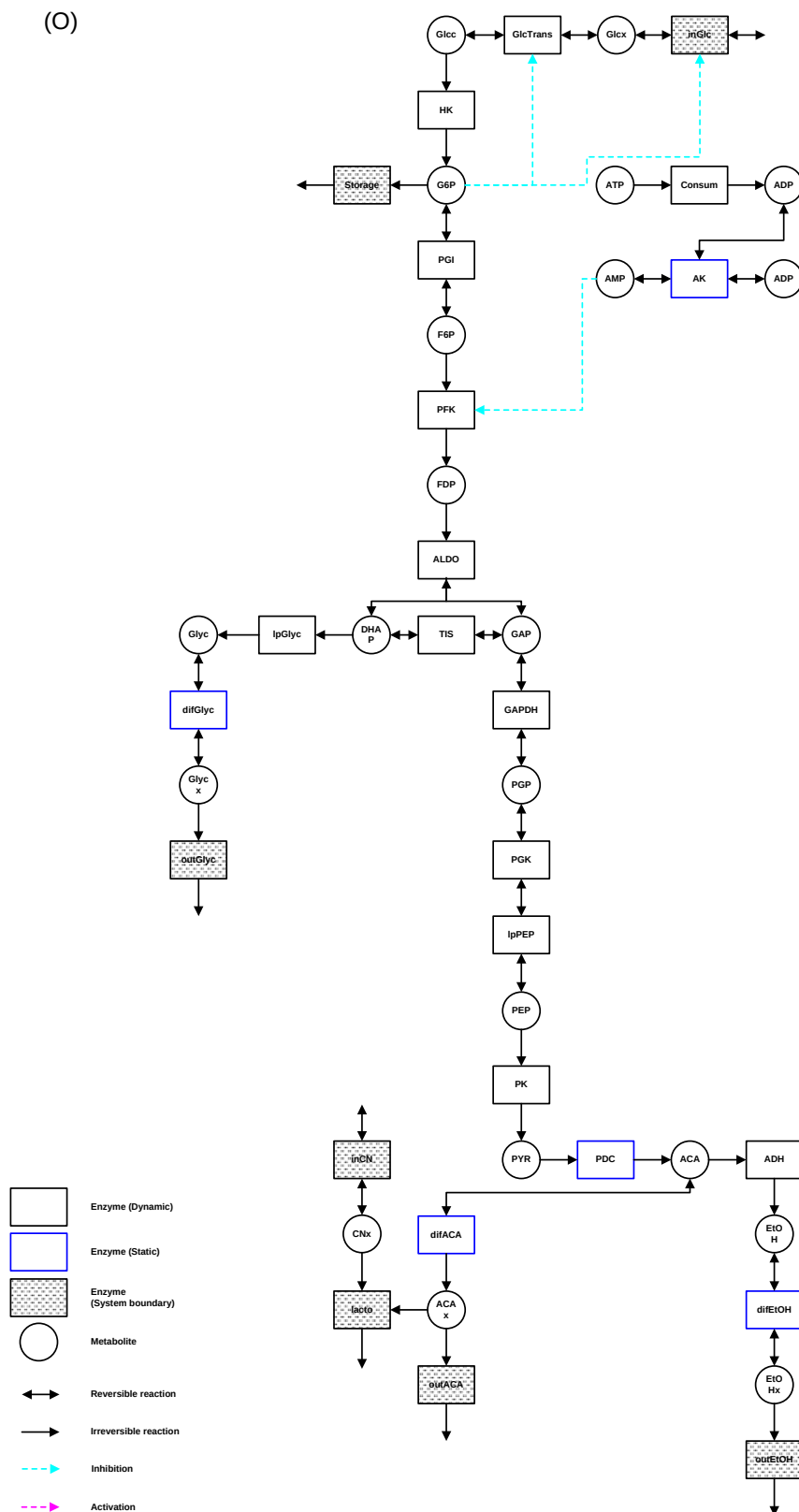


Figure S2 (Continued.)

(P)

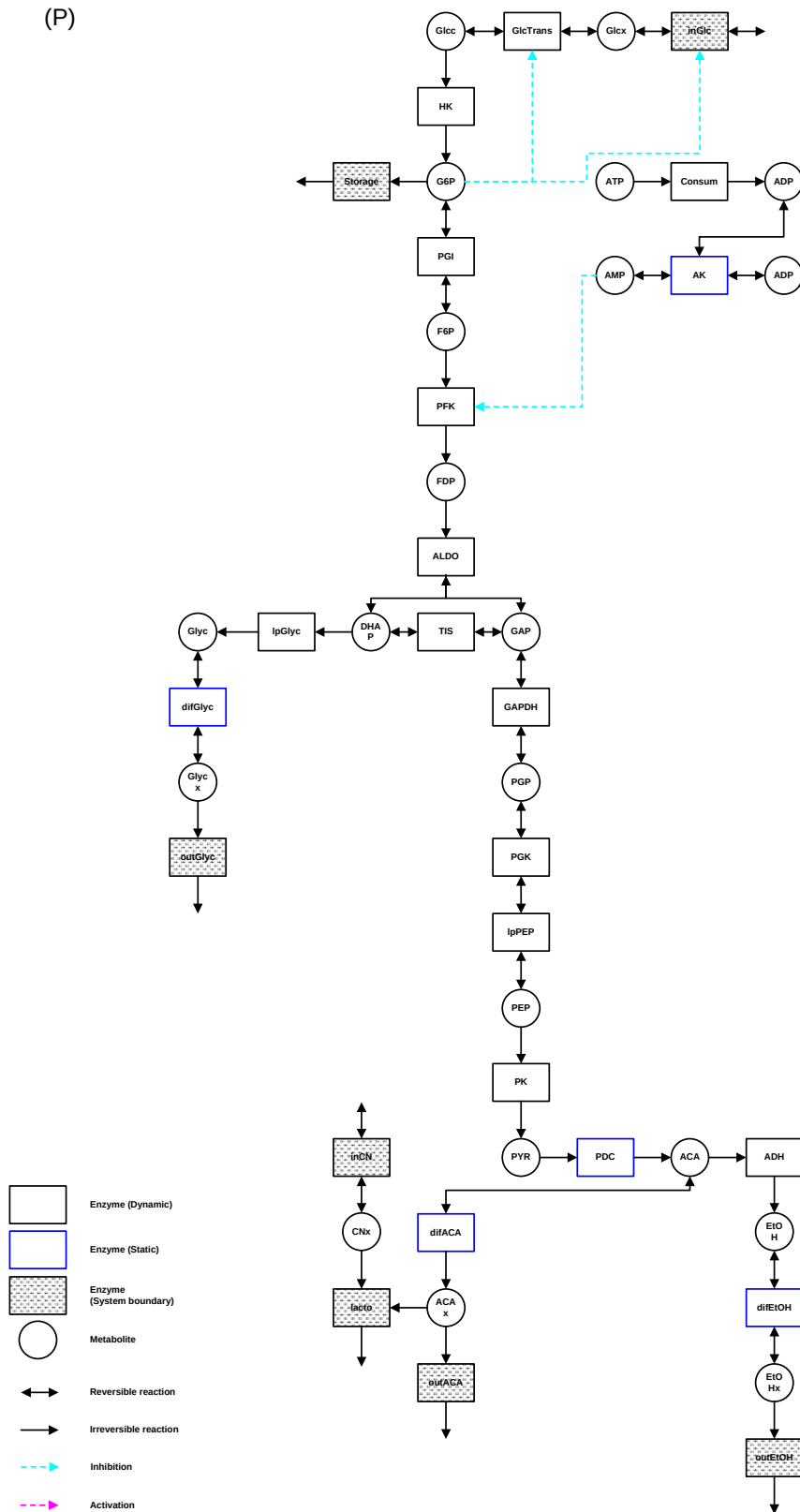


Figure S2 (Continued.)

(Q)

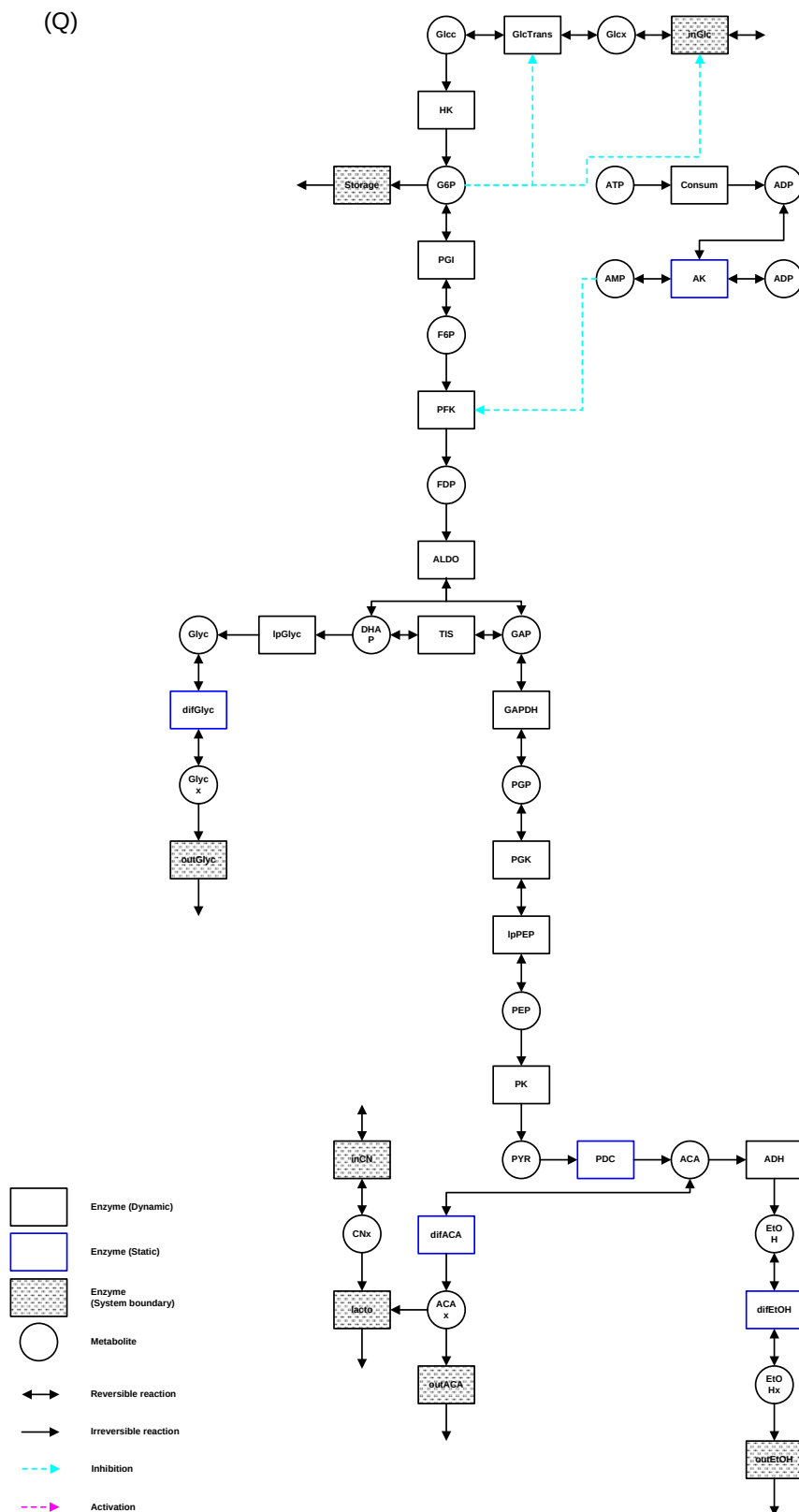


Figure S2 (Continued.)

(R)

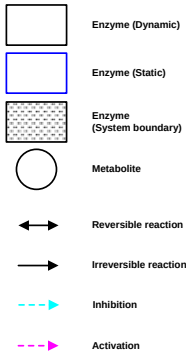


Figure S2 (Continued.)

Figure S2 Dynamic/static distinctions displayed on the metabolic map. (A) *E. coli*, weighting coefficient (w) = 1.000; (B) *E. coli*, w = 0.750; (C) *E. coli*, w = 0.500; (D) *E. coli*, w = 0.250; (E) *E. coli*, w = 0.100; (F) *E. coli*, w = 0.075; (G) *E. coli*, w = 0.050; (H) *E. coli*, w = 0.025; (I) *E. coli*, w = 0.010; (J) *S. cerevisiae*, w = 1.000; (K) *S. cerevisiae*, w = 0.750; (L) *S. cerevisiae*, w = 0.500; (M) *S. cerevisiae*, w = 0.250; (N) *S. cerevisiae*, w = 0.100; (O) *S. cerevisiae*, w = 0.075; (P) *S. cerevisiae*, w = 0.050; (Q) *S. cerevisiae*, w = 0.025; (R) *S. cerevisiae*, w = 0.010.

(A)

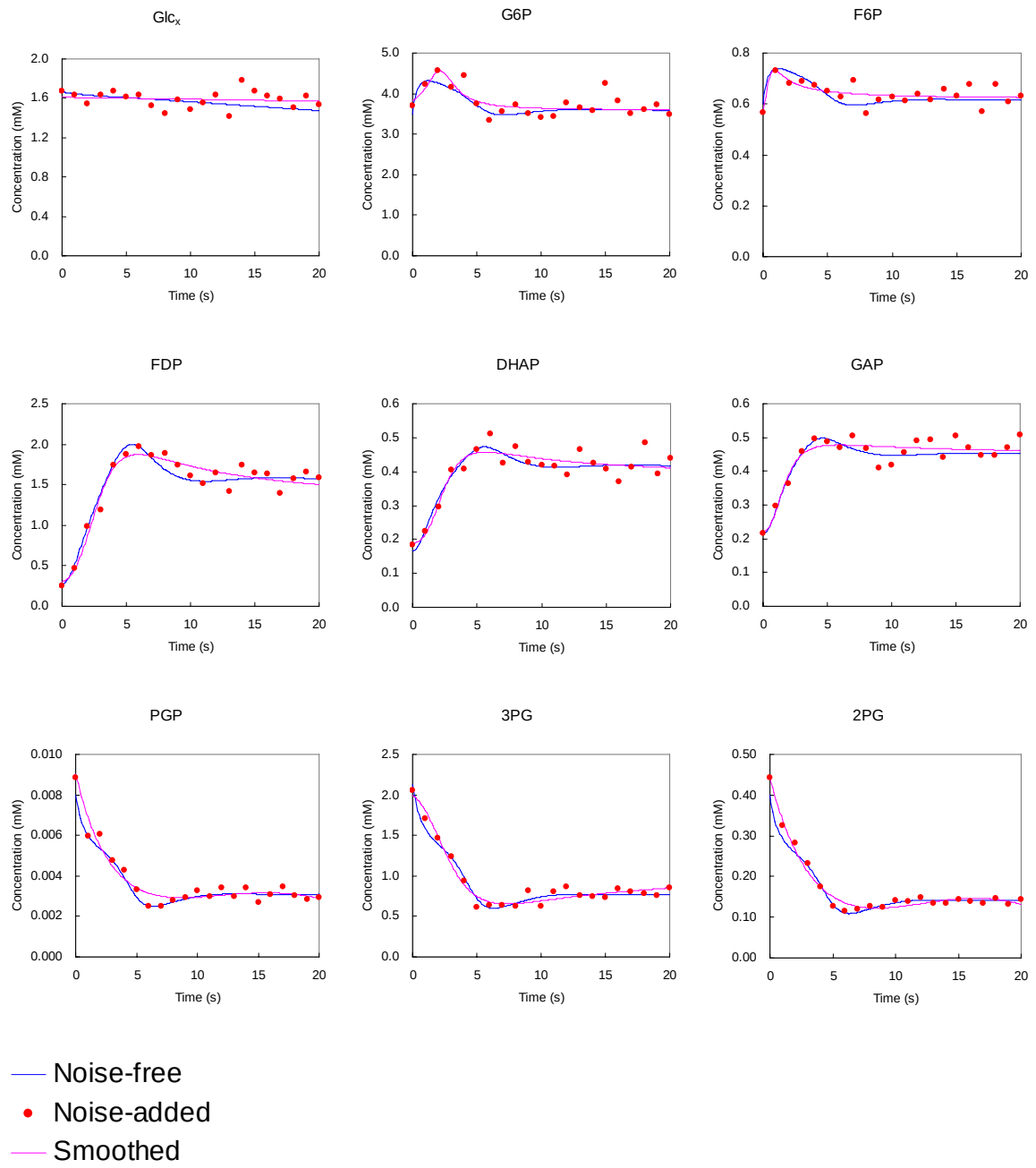


Figure S3

(B)

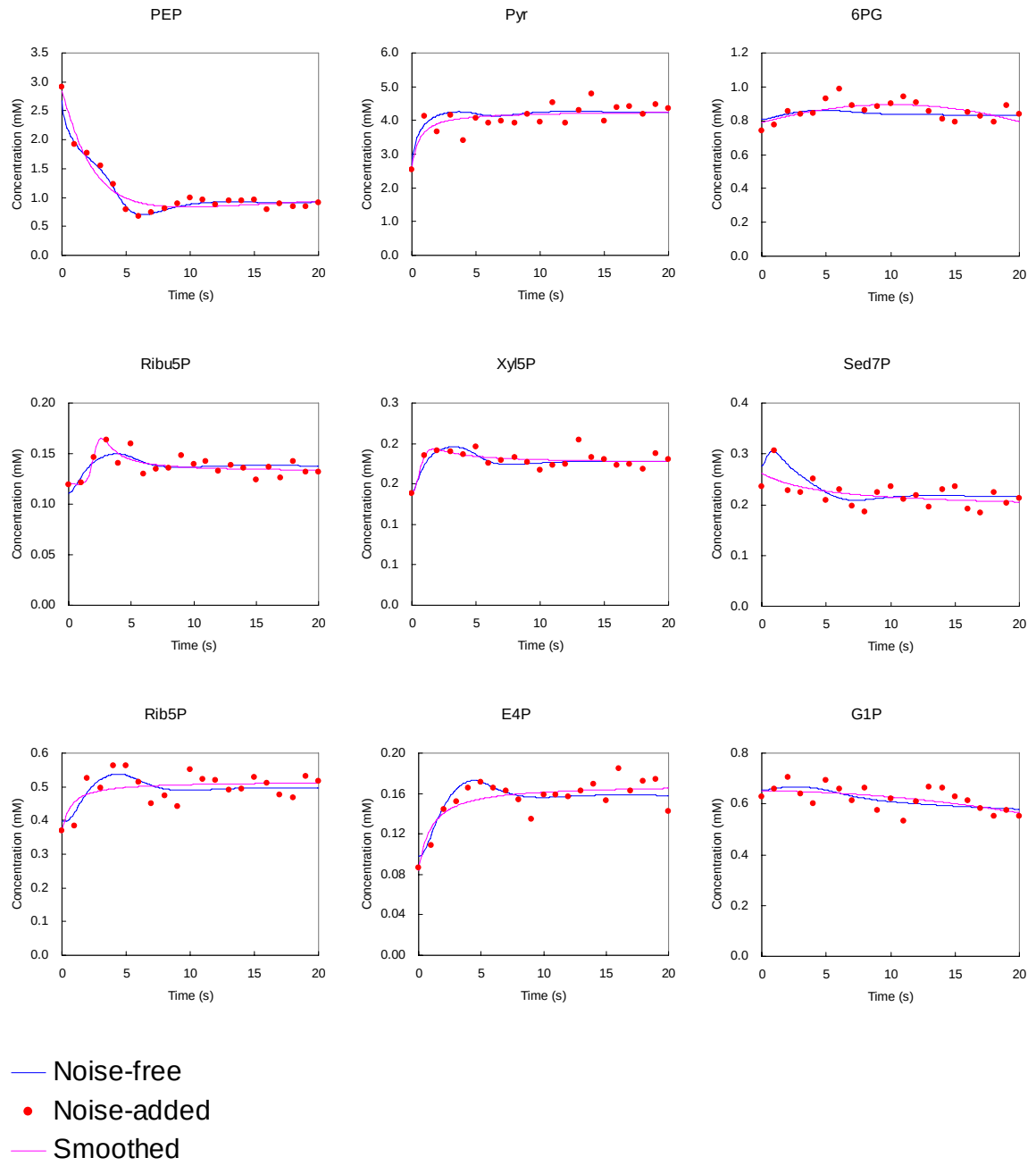


Figure S3 (Continued.)

(C)

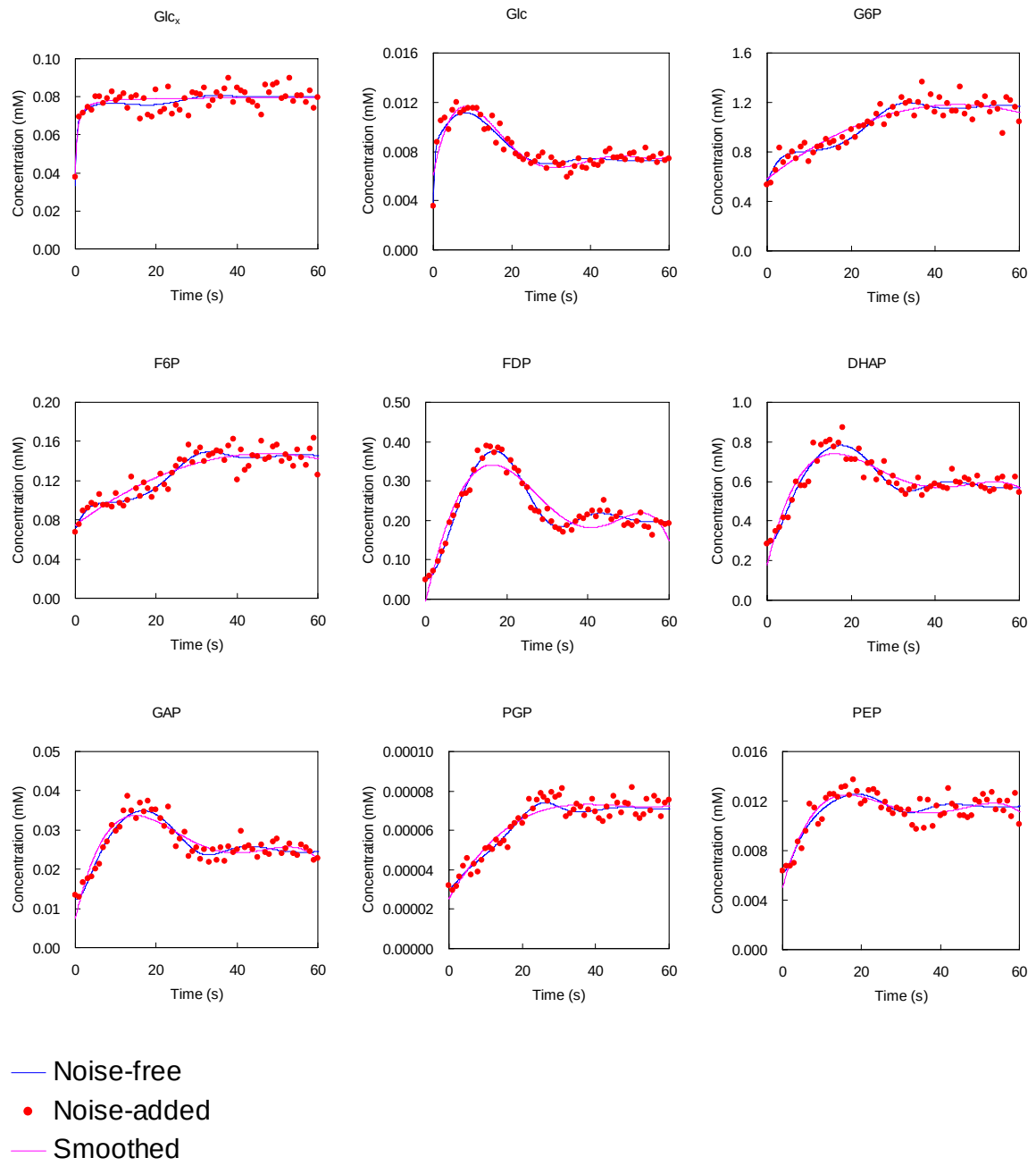


Figure S3 (Continued.)

(D)

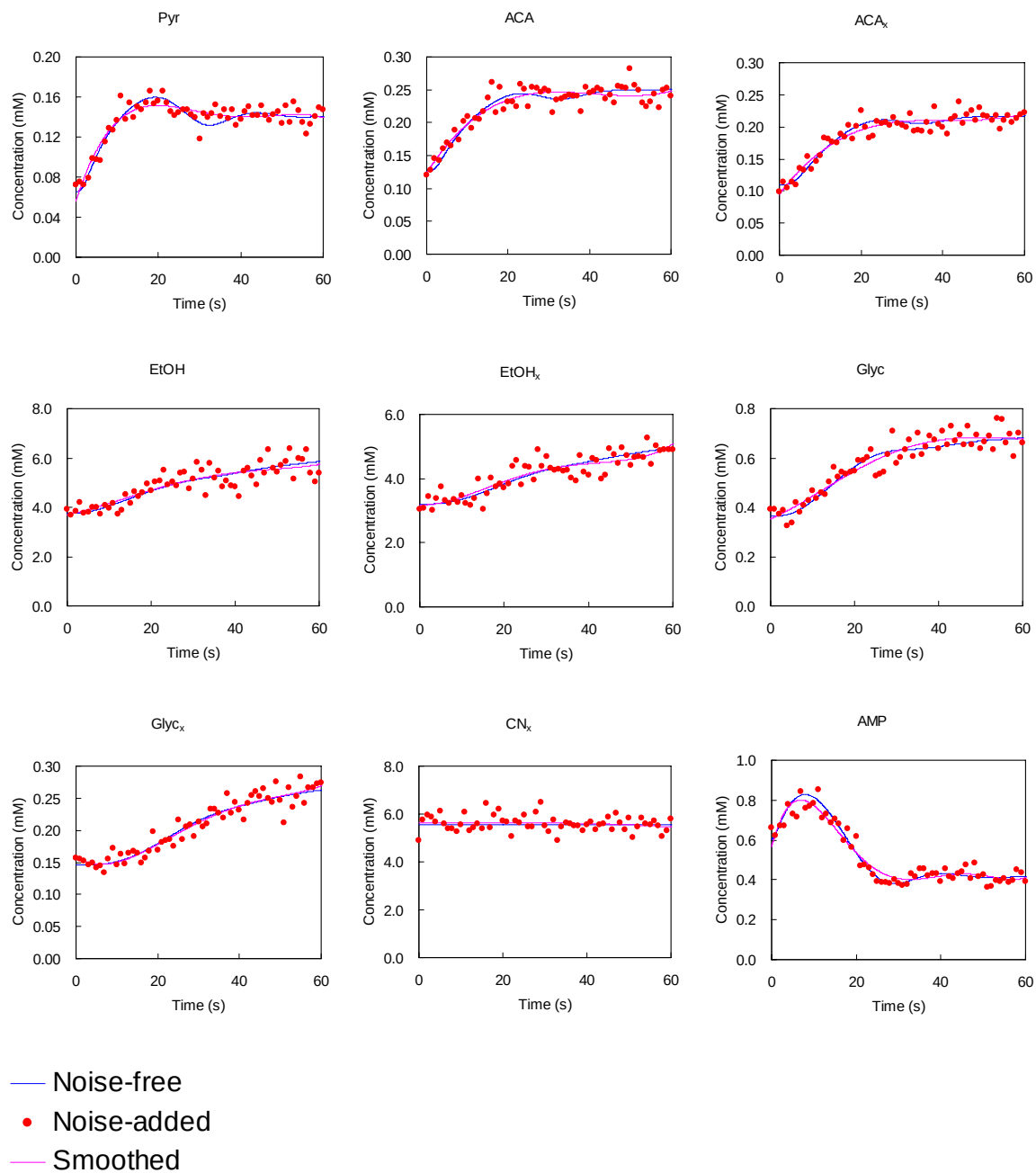


Figure S3 (Continued.)

(E)

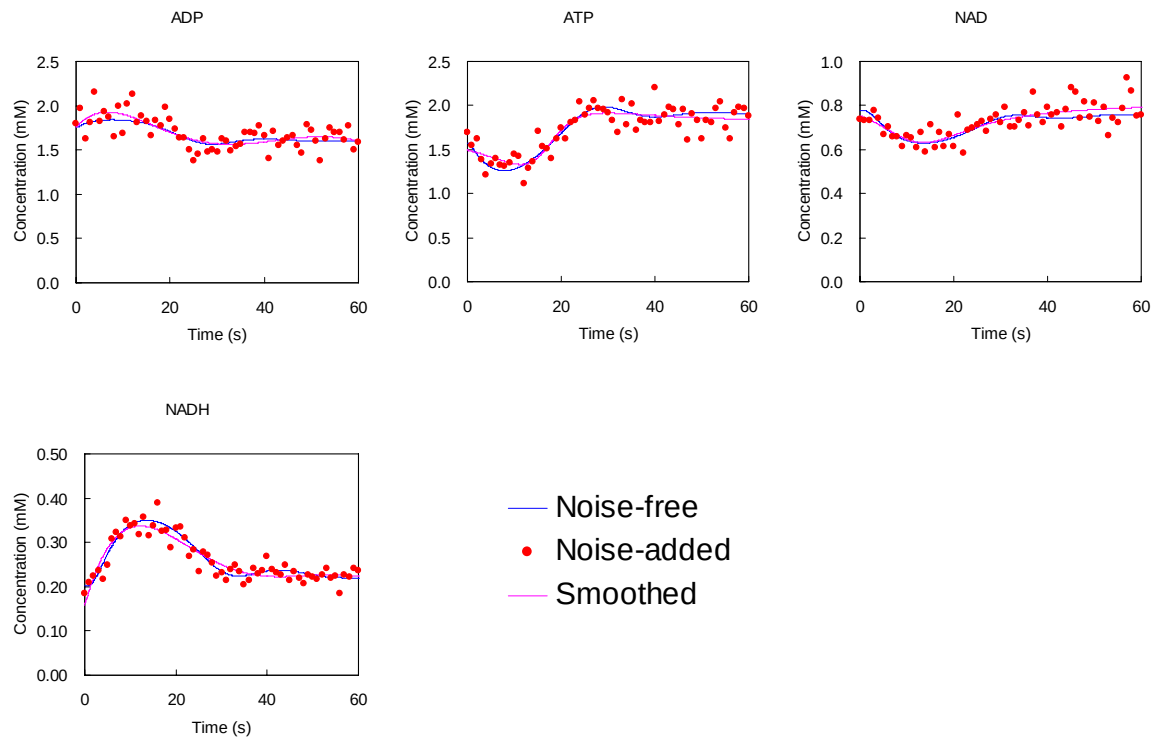


Figure S3 Noise-free, noise-added, and smoothed metabolite concentration time series.

(A) and (B), *E. coli*; (C), (D), and (E), *S. cerevisiae*. Each noise-added data point is the average of five noise-added values.