1 Branch point point model Simulations with alpha = 0, beta = 0

### Branch point model with artificial data (noise-free kinetic data, noise-free state data) - alpha = 0, beta = 0

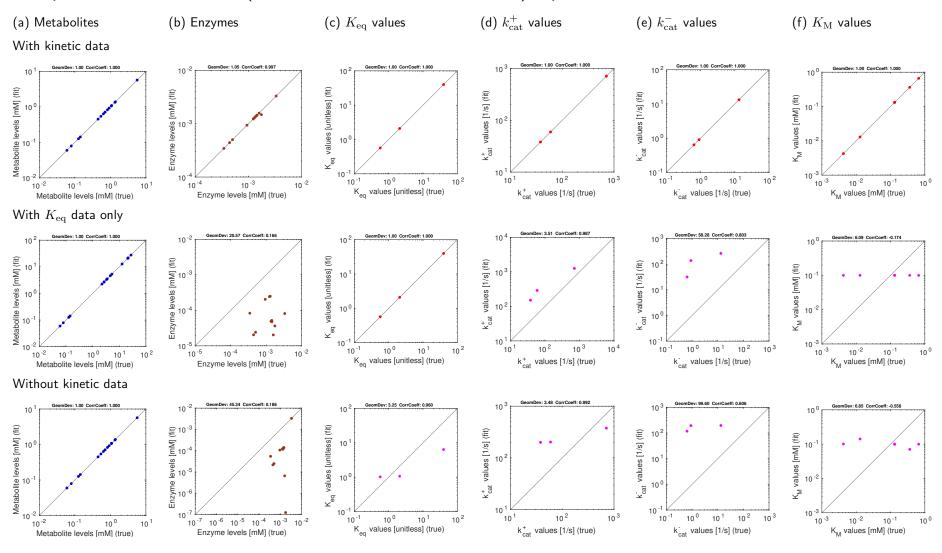


Figure 3: Model balancing results for Branch point model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation coefficients ("CorrCoeff"). (a) Metabolite levels. (b) Enzyme levels. (c)-(f) Different types of kinetic constants. Rows show different estimation scenarios (see Figure) Upper row: simple scenario S1 (noise-free artificial data, data for kinetic constants). Centre row: scenario S2 (noise-free artificial data, no data for kinetic constants). Depending on the scenario, kinetic constants are either fitted (red dots) or predicted (magenta dots).

### Branch point model with artificial data (noisy kinetic data, noise-free state data) - alpha = 0, beta = 0

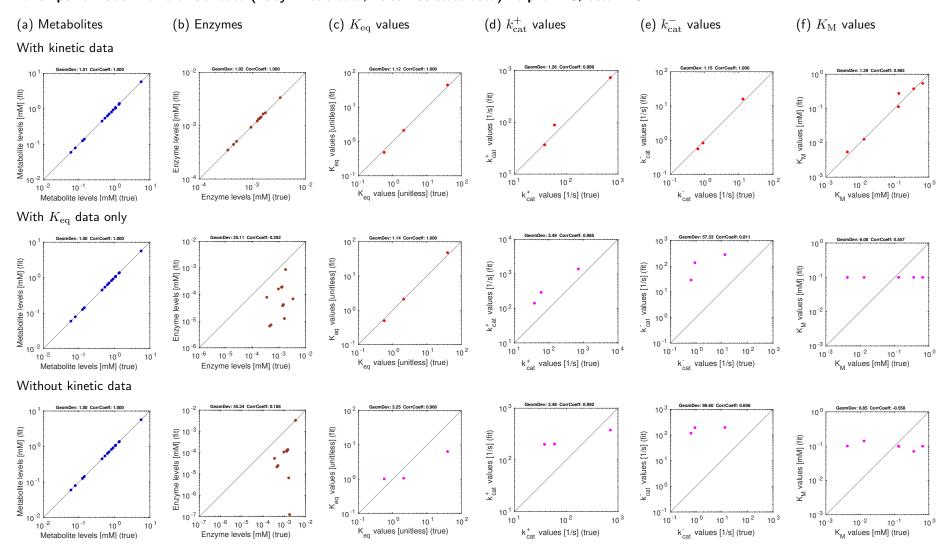


Figure 4: Same as Figure 8, with noisy kinetic data

## Branch point model with artificial data (noise-free kinetic data, noisy state data) - alpha = 0, beta = 0

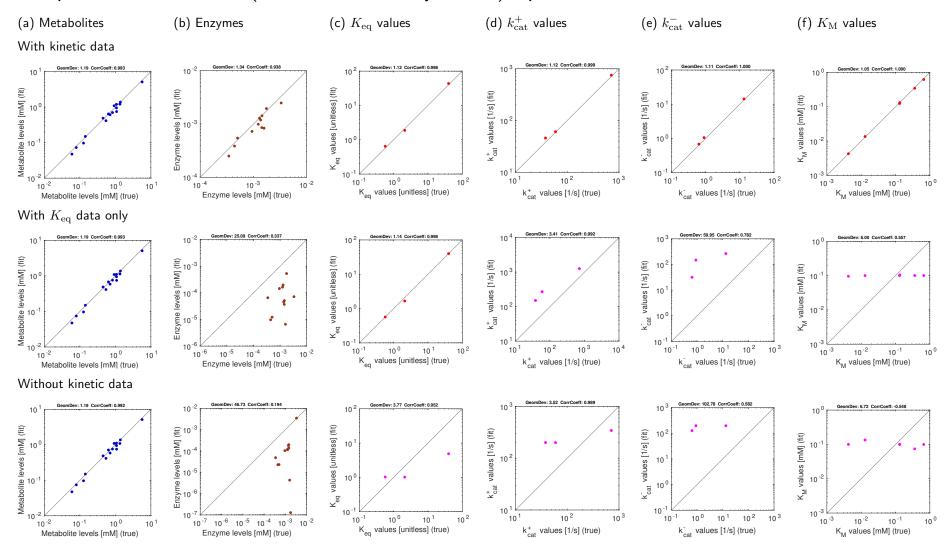


Figure 5: Results for Branch point with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial data, no data for kinetic constants).

#### Branch point model with artificial data (noisy kinetic data, noisy state data) - alpha = 0, beta = 0(c) $K_{ m eq}$ values (d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (f) $K_{ m M}$ values With kinetic data GeomDev: 1.22 CorrCoeff: 1.000 GeomDev: 1.19 CorrCoeff: 0.999 102 GeomDev: 1.28 CorrCoeff: 0.996 10 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) Enzyme levels [mM] (fit) values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>1</sup> 10 10<sup>2</sup> 10 <sup>0</sup> 10<sup>0</sup> 10 <sup>-2</sup> + cat , zat 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10<sup>0</sup> K<sub>eq</sub> values [unitless] (true) k + values [1/s] (true) k<sub>cat</sub> values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) With $K_{\mathrm{eq}}$ data only GeomDev: 62.62 CorrCoeff: 0.726 GeomDev: 24.05 CorrCoeff: 0.433 GeomDev: 1.52 CorrCoeff: 0.991 10<sup>3</sup> GeomDev: 6.04 CorrCoeff: 0.557 10 10-2 10 levels [mM] (fit) (fit) 10 -4 values [unitless] (fit) values [1/s] (fit) values [1/s] (fit) 10<sup>2</sup> values [mM] (fit) 10 10<sup>2</sup> 10 <sup>1</sup> 10 <sup>0</sup> Metabolite Io 10<sup>0</sup> , cat ⊼ 8 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-1</sup> 10<sup>3</sup> 10 <sup>0</sup> 10 <sup>1</sup> 10<sup>2</sup> 10 <sup>-3</sup> 10<sup>0</sup> 10<sup>-6</sup> 10<sup>-5</sup> 10<sup>-4</sup> 10<sup>-3</sup> 10 <sup>0</sup> 10 <sup>1</sup> K<sub>eq</sub> values [unitless] (true) k<sub>cat</sub> values [1/s] (true) k at values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data GeomDev: 46.73 CorrCoeff: 0.194 GeomDev: 3.77 CorrCoeff: 0.952 GeomDev: 6.72 CorrCoeff: -0.548 10 10 10 -2 10 2 Metabolite levels [mM] (fit) (iti) [mM] (iti) 10<sup>-3</sup> values [unitless] (fit) values [1/s] (fit) values [mM] (fit) values [1/s] (fit) 10<sup>2</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10 <sup>0</sup> (at 10°) κ cat

Figure 6: Same as Figure 10, with noisy kinetic data

10<sup>1</sup>

K<sub>eq</sub> values [unitless] (true)

10<sup>-7</sup> 10<sup>-6</sup> 10<sup>-5</sup> 10<sup>-4</sup> 10<sup>-3</sup> 10<sup>-2</sup>

Enzyme levels [mM] (true)

10 <sup>0</sup>

10 <sup>-1</sup>

Metabolite levels [mM] (true)

10<sup>1</sup>

10<sup>1</sup>

10<sup>2</sup>

k + values [1/s] (true)

10<sup>3</sup>

10<sup>-1</sup>

10<sup>2</sup>

10 <sup>-3</sup>

10<sup>-2</sup>

K<sub>M</sub> values [mM] (true)

10 <sup>0</sup>

10 <sup>1</sup>

k-at values [1/s] (true)

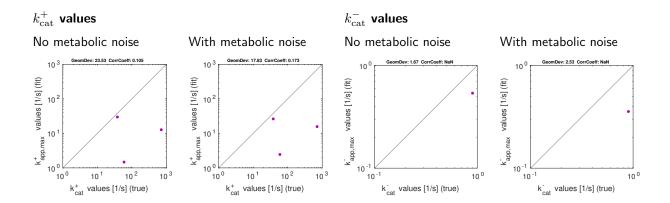


Figure 7: Catalytic constants in Branch point (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).

2 Branch point point model Simulations with alpha = 1, beta = 0

### Branch point model with artificial data (noise-free kinetic data, noise-free state data) - alpha = 1, beta = 0

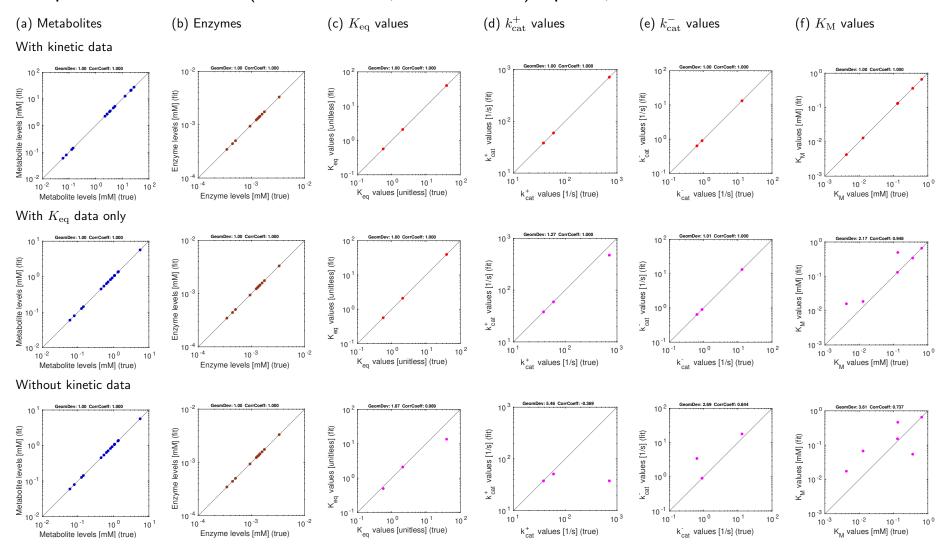


Figure 8: Model balancing results for Branch point model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation coefficients ("CorrCoeff"). (a) Metabolite levels. (b) Enzyme levels. (c)-(f) Different types of kinetic constants. Rows show different estimation scenarios (see Figure) Upper row: simple scenario S1 (noise-free artificial data, data for kinetic constants). Centre row: scenario S2 (noise-free artificial data, no data for kinetic constants). Depending on the scenario, kinetic constants are either fitted (red dots) or predicted (magenta dots).

10<sup>-2</sup>

10 <sup>-1</sup>

Metabolite levels [mM] (true)

10 <sup>0</sup>

10<sup>1</sup>

10 -4

10<sup>-4</sup>

10 <sup>-3</sup>

Enzyme levels [mM] (true)

10 <sup>-2</sup>

#### Branch point model with artificial data (noisy kinetic data, noise-free state data) - alpha = 1, beta = 0(c) $K_{ m eq}$ values (d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (f) $K_{ m M}$ values With kinetic data GeomDev: 1.25 CorrCoeff: 0.990 GeomDev: 1.22 CorrCoeff: 0.999 10 102 GeomDev: 1.39 CorrCoeff: 0.985 10<sup>2</sup> 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) Enzyme levels [mM] (fit) 0 6 values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>1</sup> 10 10<sup>2</sup> 10<sup>0</sup> 10<sup>0</sup> 10<sup>0</sup> 10 <sup>-2</sup> + cat , zat 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10 <sup>-1</sup> 10<sup>0</sup> 10 <sup>1</sup> K<sub>eq</sub> values [unitless] (true) k + values [1/s] (true) k<sub>cat</sub> values [1/s] (true) K<sub>M</sub> values [mM] (true) Enzyme levels [mM] (true) Metabolite levels [mM] (true) With $K_{\mathrm{eq}}$ data only GeomDev: 1.24 CorrCoeff: 0.999 10 10<sup>2</sup> GeomDev: 2.18 CorrCoeff: 0.943 10 10-2 10 Metabolite levels [mM] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>1</sup> 10 10<sup>2</sup> 10<sup>0</sup> 10 <sup>0</sup> , cat 10 -10<sup>2</sup> 10<sup>3</sup> 10 <sup>0</sup> 10<sup>-1</sup> 10 <sup>1</sup> 10<sup>2</sup> 10 <sup>0</sup> 10 <sup>1</sup> 10<sup>2</sup> 10 <sup>-3</sup> 10<sup>0</sup> 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10 <sup>-1</sup> 10 <sup>0</sup> 10 <sup>1</sup> K<sub>eq</sub> values [unitless] (true) k + values [1/s] (true) k at values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data Dev: 1.00 CorrCoeff: 1.000 GeomDev: 1.87 CorrCoeff: 0.989 GeomDev: 2.69 CorrCoeff: 0.844 GeomDev: 3.61 CorrCoeff: 0.737 102 10 10 -2 10 2 Metabolite levels [mM] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>1</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>0</sup> 10 <sup>0</sup> , cat

Figure 9: Same as Figure 8, with noisy kinetic data

10<sup>1</sup>

K<sub>eq</sub> values [unitless] (true)

κ cat

10<sup>1</sup>

10<sup>2</sup>

k + values [1/s] (true)

10<sup>3</sup>

10<sup>-1</sup>

10 <sup>0</sup>

k at values [1/s] (true)

10 <sup>1</sup>

10<sup>2</sup>

10 <sup>-3</sup>

10<sup>-2</sup>

K<sub>M</sub> values [mM] (true)

10 <sup>0</sup>

# Branch point model with artificial data (noise-free kinetic data, noisy state data) - alpha = 1, beta = 0

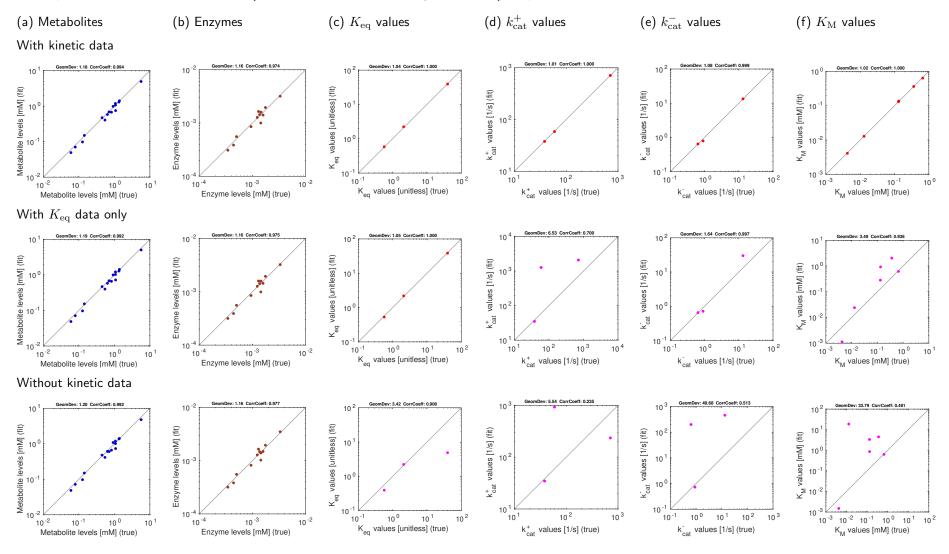


Figure 10: Results for Branch point with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial data, no data for kinetic constants).

10 -4

10 <sup>0</sup>

10 <sup>-1</sup>

Metabolite levels [mM] (true)

10<sup>1</sup>

10<sup>-4</sup>

10 <sup>-3</sup>

Enzyme levels [mM] (true)

10 <sup>-2</sup>

#### Branch point model with artificial data (noisy kinetic data, noisy state data) - alpha = 1, beta = 0(c) $K_{ m eq}$ values (d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (f) $K_{ m M}$ values With kinetic data GeomDev: 1.16 CorrCoeff: 1.000 GeomDev: 1.18 CorrCoeff: 0.993 GeomDev: 1.25 CorrCoeff: 1.000 102 GeomBey: 1.31 CorrCoeff: 0.996 10 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) Enzyme levels [mM] (fit) 0 6 values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>1</sup> 10 10<sup>2</sup> 10 <sup>0</sup> 10<sup>0</sup> 10 <sup>-2</sup> + cat , zat 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10<sup>0</sup> K<sub>eq</sub> values [unitless] (true) k + values [1/s] (true) k<sub>cat</sub> values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) With $K_{\mathrm{eq}}$ data only GeomDev: 1.68 CorrCoeff: 0.949 10 10<sup>2</sup> GeomDev: 5.08 CorrCoeff: 0.858 10 10-2 10 levels [mM] (fit) values [1/s] (fit) 0 10 2 2 Enzyme levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) 10<sup>0</sup> values [mM] (fit) 10<sup>1</sup> 10 10<sup>0</sup> 10 <sup>0</sup> Metabolite Io , cat ×8 10 " 10<sup>3</sup> 10 <sup>0</sup> 10<sup>2</sup> 10<sup>4</sup> 10<sup>-1</sup> 10 <sup>1</sup> 10<sup>2</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-2</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10 <sup>-1</sup> 10 <sup>0</sup> 10 <sup>1</sup> K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) k<sub>cat</sub> values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data nDev: 1.16 CorrCoeff: 0.977 GeomDev: 3.42 CorrCoeff: 0.908 GeomDev: 33.79 CorrCoeff: 0.481 10<sup>3</sup> 10 10 -2 10 2 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) values [1/s] (fit) 10<sup>2</sup> 10 <sup>1</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 <sup>0</sup> (at 10°) κ cat

Figure 11: Same as Figure 10, with noisy kinetic data

10<sup>1</sup>

K<sub>eq</sub> values [unitless] (true)

10<sup>1</sup>

10<sup>2</sup>

k + values [1/s] (true)

10<sup>3</sup>

10<sup>-1</sup>

10<sup>2</sup>

10-3 10-2 10-1 100 101

K<sub>M</sub> values [mM] (true)

10 <sup>1</sup>

k-at values [1/s] (true)

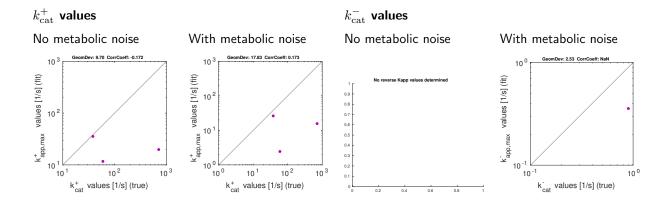


Figure 12: Catalytic constants in Branch point (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).