- 1 Double branch model Simulations with alpha = 0
- 2 Double branch point model Simulations with alpha = 0, beta = 0

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

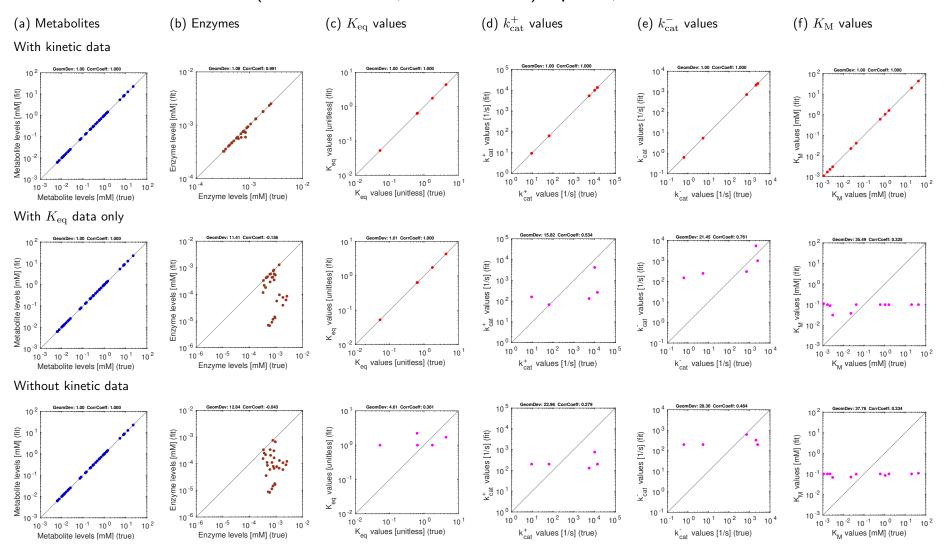


Figure 3: Model balancing results for Double branch model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation 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 S1K (noise-free artificial data, kinetic data given only for equilibrium constants). Lower 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).

### Double branch model with artificial data (noisy kinetic data, noise-free state data) - alpha = 0, beta = 0(d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.40 CorrCoeff: 0.998 10 <sup>4</sup> 10 10-2 10 values [1/s] (fit) values [unitless] (fit) 10<sup>1</sup> Metabolite levels [mM] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10 -10<sup>2</sup> 10 <sup>1</sup> 10 10 -2 + tg 10 , tg 10 ℃ 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10 <sup>-2</sup> 10<sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>0</sup> 10 <sup>1</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> 10<sup>2</sup> 10<sup>-5</sup> 10 <sup>-4</sup> 10<sup>-3</sup> 10-3 10-2 10-1 100 101 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) Enzyme levels [mM] (true) Metabolite levels [mM] (true) With $K_{\rm eq}$ data only GeomDev: 15.11 CorrCoeff: 0.563 GeomDev: 21.27 CorrCoeff: 0.676 10 10-10 4 10 values [1/s] (fit) levels [mM] (fit) values [unitless] (fit) 10<sup>3</sup> values [1/s] (fit) values [mM] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>-1</sup> + tg 10 , jg 10 g 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10-3 10-2 10-1 100 101 102 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-6</sup> 10<sup>-5</sup> 10<sup>-4</sup> 10<sup>-3</sup> k at values [1/s] (true) $k_{cat}^+$ values [1/s] (true) K<sub>M</sub> values [mM] (true) Ken values [unitless] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data GeomDev: 28.36 CorrCoeff: 0.484 GeomDev: 12.84 CorrCoeff: -0.043 10 10 GeomDev: 37.78 CorrCoeff: 0.334 10<sup>2</sup> 10-2 10 values [1/s] (fit) levels [mM] (fit) Metabolite levels [mM] (fit) values [unitless] (fit) 10<sup>3</sup> values [mM] (fit) values [1/s] (fit)

Figure 4: Same as Figure 13, with noisy kinetic data

10 <sup>1</sup>

10 <sup>0</sup>

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

10<sup>3</sup>

10<sup>2</sup>

10 <sup>1</sup>

10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

k<sub>cat</sub> values [1/s] (true)

10 gt +

10<sup>2</sup>

10 <sup>1</sup>

10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup>

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

`å 10 g

10<sup>0</sup>

10<sup>-3</sup> 10<sup>-2</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup>

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

10<sup>0</sup>

10

10<sup>-6</sup> 10 <sup>-5</sup>

10 -3 10 -2 10 -1 10 0 10 1

Metabolite levels [mM] (true)

10 <sup>-4</sup> 10 <sup>-3</sup>

Enzyme levels [mM] (true)

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

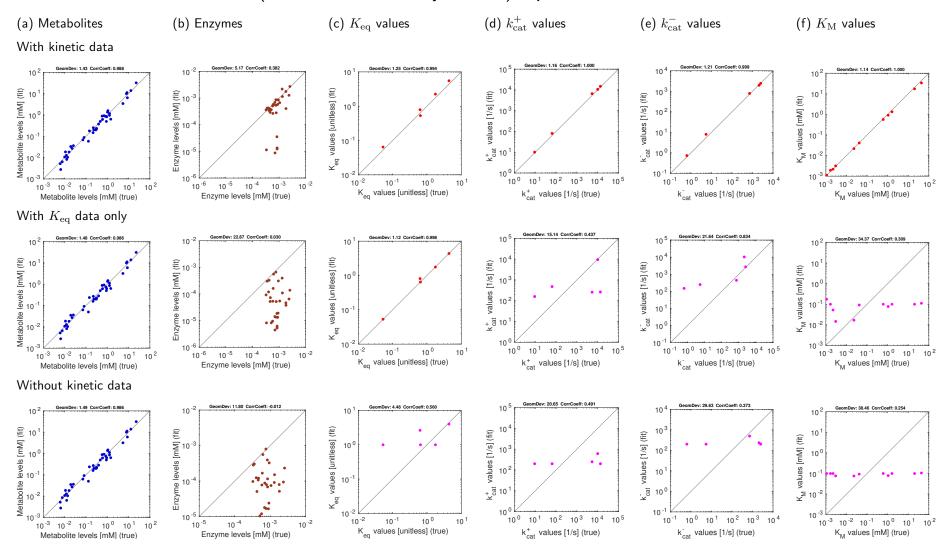


Figure 5: Results for Double branch with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial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

### Double branch model with artificial data (noisy kinetic data, noisy state data) - alpha = 0, beta = 0(d) $k_{\rm cat}^+$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (e) $k_{\rm cat}^-$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.62 CorrCoeff: 0.994 10 <sup>4</sup> 10 10-2 values [1/s] (fit) levels [mM] (fit) values [unitless] (fit) Metabolite levels [mM] (fit) 103 values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 + tg 10 , tg 10 ℃ . ≥ 10 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10 <sup>-2</sup> 10<sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>0</sup> 10 <sup>1</sup> 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-6</sup> 10 <sup>-5</sup> 10 <sup>-4</sup> 10<sup>-3</sup> 10-3 10-2 10-1 100 101 K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) k+ values [1/s] (true) K<sub>M</sub> values [mM] (true) Enzyme levels [mM] (true) Metabolite levels [mM] (true) With $K_{\rm eq}$ data only GeomDev: 15.10 CorrCoeff: 0.502 GeomDev: 23.35 CorrCoeff: 0.765 GeomDev: 1.55 CorrCoeff: 0.957 10 10-10 4 10 values [1/s] (fit) levels [mM] (fit) values [unitless] (fit) 10<sup>3</sup> values [1/s] (fit) values [mM] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>-1</sup> + tg 10 , jg 10 g 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10-3 10-2 10-1 100 101 102 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-6</sup> 10<sup>-5</sup> 10<sup>-4</sup> 10<sup>-3</sup> k at values [1/s] (true) K<sub>eq</sub> values [unitless] (true) $k_{cat}^+$ values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data GeomDev: 29.63 CorrCoeff: 0.373 GeomDev: 11.80 CorrCoeff: -0.012 10 10 10<sup>2</sup> 10 -2 10 Metabolite levels [mM] (fit) Enzyme levels [mM] (fit) values [1/s] (fit) values [unitless] (fit) 10<sup>3</sup> values [mM] (fit) values [1/s] (fit) 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 10 gt `å 10 g 10 <sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup>

Figure 6: Same as Figure 15, with noisy kinetic data

k<sub>cat</sub> values [1/s] (true)

10<sup>-3</sup> 10<sup>-2</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup>

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

k at values [1/s] (true)

10 <sup>1</sup>

10 <sup>0</sup>

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

10 <sup>-4</sup>

Enzyme levels [mM] (true)

10<sup>-5</sup>

10 -3 10 -2 10 -1 10 0 10 1

Metabolite levels [mM] (true)

10<sup>-3</sup>

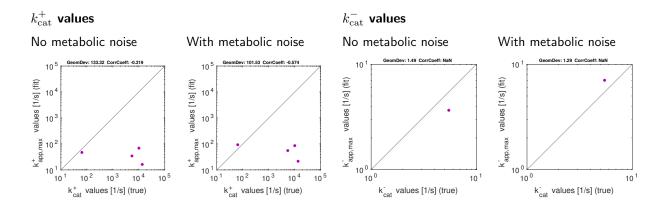


Figure 7: Catalytic constants in Double branch (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).

- 3 Double branch model Simulations with alpha = 0.1
- 4 Double branch point model Simulations with alpha = 0.1, beta = 0

## Double branch model with artificial data (noise-free kinetic data, noise-free state data) - alpha = 0.1, beta = 0

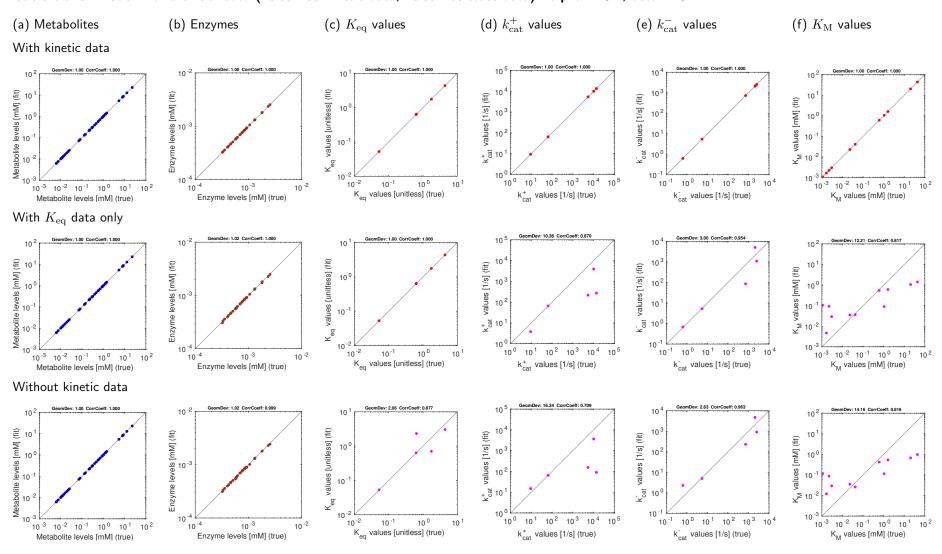


Figure 8: Model balancing results for Double branch model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation 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 S1K (noise-free artificial data, kinetic data given only for equilibrium constants). Lower 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 -3 10 -2 10 -1 10 0 10 1

Metabolite levels [mM] (true)

### Double branch model with artificial data (noisy kinetic data, noise-free state data) - alpha = 0.1, beta = 0(d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.35 CorrCoeff: 0.999 10 <sup>4</sup> 10 10-2 10 values [1/s] (fit) values [unitless] (fit) 10<sup>1</sup> Metabolite levels [mM] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 + tg 10 , tg 10 °C 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10 <sup>-2</sup> 10 <sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>0</sup> 10 <sup>1</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> 10<sup>2</sup> 10-3 10-2 10-1 100 101 K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) k+ values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) With $K_{\rm eq}$ data only GeomDev: 10.89 CorrCoeff: 0.790 GeomDev: 13.92 CorrCoeff: 0.760 GeomDev: 1.37 CorrCoeff: 0.982 10 10 4 10-10 Metabolite levels [mM] (fit) values [1/s] (fit) values [unitless] (fit) 10<sup>3</sup> Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>1</sup> 10 + tg 10 , jg 10 g 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10 <sup>1</sup> 10-3 10-2 10-1 100 101 102 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> k at values [1/s] (true) K<sub>eq</sub> values [unitless] (true) $k_{cat}^+$ values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data GeomDev: 2.63 CorrCoeff: 0.963 10 10 GeomDev: 14.16 CorrCoeff: 0.816 10<sup>2</sup> 10-2 10 Metabolite levels [mM] (fit) values [1/s] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) 10<sup>3</sup> values [mM] (fit) values [1/s] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 10 gt + `å 10 g

Figure 9: Same as Figure 13, with noisy kinetic data

10 <sup>1</sup>

10 <sup>0</sup>

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

10 <sup>-3</sup>

Enzyme levels [mM] (true)

10<sup>-4</sup>

10 <sup>-2</sup>

10 <sup>1</sup>

10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

k<sub>cat</sub> values [1/s] (true)

10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup>

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

10<sup>-3</sup> 10<sup>-2</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup>

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

## Double branch model with artificial data (noise-free kinetic data, noisy state data) - alpha = 0.1, beta = 0

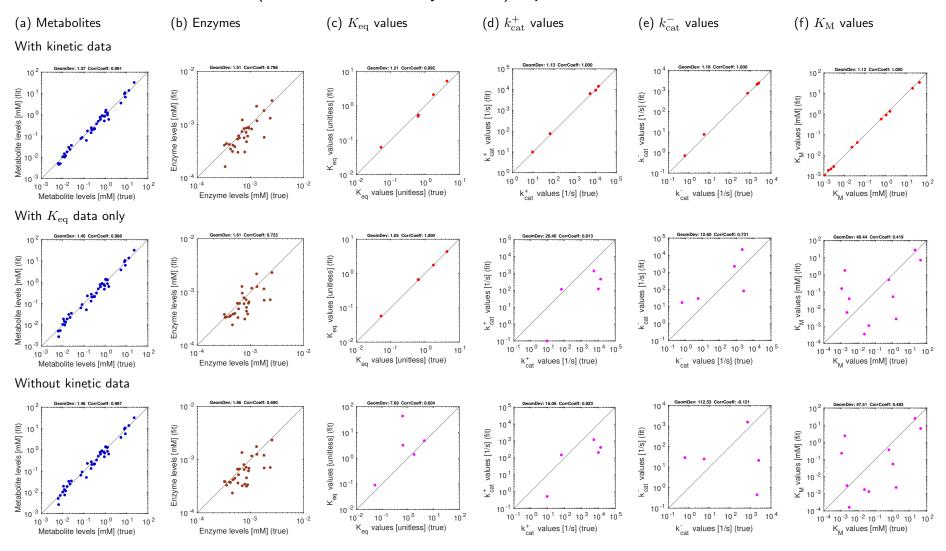


Figure 10: Results for Double branch with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial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

10 -3 10 -2 10 -1 10 0 10 1

Metabolite levels [mM] (true)

Enzyme levels [mM] (true)

#### Double branch model with artificial data (noisy kinetic data, noisy state data) - alpha = 0.1, beta = 0(d) $k_{\rm cat}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.56 CorrCoeff: 0.994 10 <sup>4</sup> 10 10-2 10 values [1/s] (fit) values [unitless] (fit) Metabolite levels [mM] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 + tg 10 , tg 10 ℃ . ≥ 10 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>-2</sup> 10<sup>0</sup> 10 <sup>1</sup> 10 -3 10 -2 10 -1 10 0 10 1 10 <sup>-4</sup> 10<sup>-3</sup> 10-3 10-2 10-1 100 101 K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) k+ values [1/s] (true) K<sub>M</sub> values [mM] (true) Enzyme levels [mM] (true) Metabolite levels [mM] (true) With $K_{\rm eq}$ data only GeomDev: 33.01 CorrCoeff: 0.135 GeomDev: 1.65 CorrCoeff: 0.708 GeomDev: 1.56 CorrCoeff: 0.956 GeomDev: 28.09 CorrCoeff: 0.801 10 10 4 10 10 values [unitless] (fit) 10<sup>3</sup> Enzyme levels [mM] (fit) values [1/s] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>3</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>2</sup> 10 <sup>-1</sup> 10 <sup>1</sup> 10 <sup>1</sup> 10<sup>-1</sup> 10 <sup>-2</sup> Metabolite I , tg 10 ° , cat 10 0 10 1 10 2 10 3 10 4 10 5 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>0</sup> 10<sup>-1</sup> 10 <sup>1</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> 10<sup>2</sup> 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-4</sup> 10 <sup>-3</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 GeomDev: 16.06 CorrCoeff: 0.823 GeomDev: 47.51 CorrCoeff: 0.483 104 10<sup>-2</sup> 10 10 10 10 Metabolite levels [mM] (fit) values [unitless] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) 10 values [1/s] (fit) values [mM] (fit) 10<sup>3</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>1</sup> 10 <sup>1</sup> 10 <sup>-2</sup> 10 و 10 g +<sup>7</sup>g 10 c

Figure 11: Same as Figure 15, with noisy kinetic data

10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

k+ values [1/s] (true)

10 <sup>1</sup>

10<sup>0</sup>

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

10<sup>3</sup>

10 -4 10 -3 10 -2 10 -1 10 0 10 1 10 2

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

10<sup>1</sup> 10<sup>2</sup>

k<sub>cat</sub> values [1/s] (true)

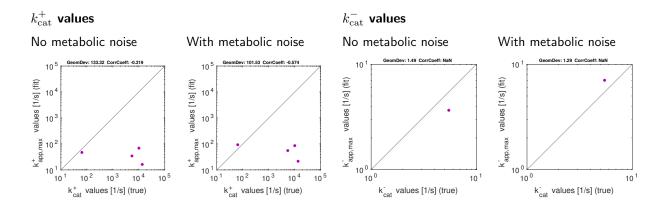


Figure 12: Catalytic constants in Double branch (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).

- 5 Double branch model Simulations with alpha = 1
- 6 Double branch point model Simulations with alpha = 1, beta = 0

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

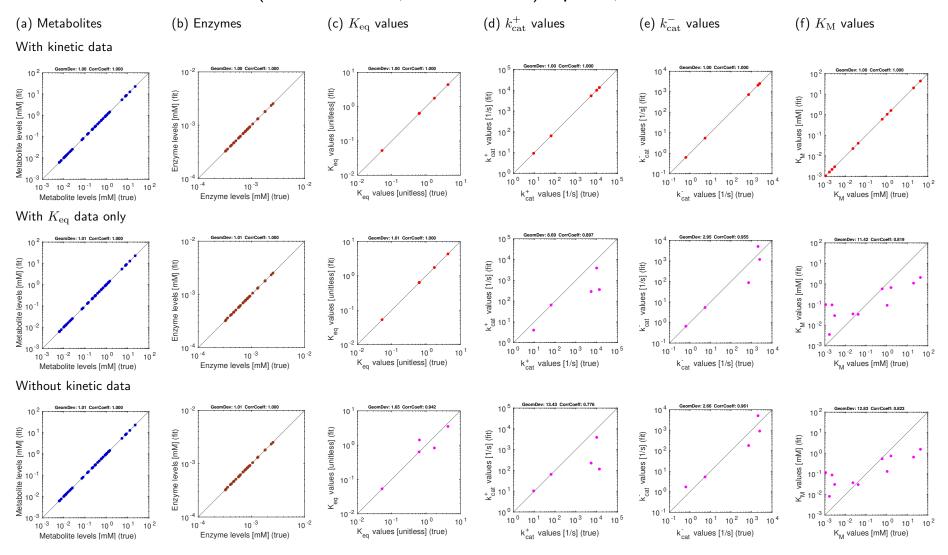


Figure 13: Model balancing results for Double branch model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation 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 S1K (noise-free artificial data, kinetic data given only for equilibrium constants). Lower 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 -3 10 -2 10 -1 10 0 10 1

Metabolite levels [mM] (true)

### Double branch model with artificial data (noisy kinetic data, noise-free state data) - alpha = 1, beta = 0(d) $k_{\text{cat}}^+$ values (e) $k_{\rm cat}^-$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.33 CorrCoeff: 0.999 10 <sup>4</sup> 10 10-2 10 values [1/s] (fit) values [unitless] (fit) 10<sup>1</sup> Metabolite levels [mM] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 + tg 10 , tg 10 ℃ 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10 <sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>-2</sup> 10<sup>0</sup> 10 <sup>1</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> 10<sup>2</sup> 10 <sup>-3</sup> 10-3 10-2 10-1 100 101 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) With $K_{\rm eq}$ data only GeomDev: 9.47 CorrCoeff: 0.827 GeomDev: 14.11 CorrCoeff: 0.759 GeomDev: 1.37 CorrCoeff: 0.983 10 10 4 10-10 values [1/s] (fit) values [unitless] (fit) 10<sup>3</sup> Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10<sup>1</sup> 10 + tg 10 , jg 10 g 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>0</sup> 10 <sup>1</sup> 10-3 10-2 10-1 100 101 102 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-4</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> k at values [1/s] (true) K<sub>eq</sub> values [unitless] (true) $k_{cat}^+$ values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data GeomDev: 13.43 CorrCoeff: 0.776 GeomDev: 2.66 CorrCoeff: 0.961 GeomDev: 1.65 CorrCoeff: 0.942 10 10 GeomDev: 12.83 CorrCoeff: 0.823 10<sup>2</sup> 10-2 10 Metabolite levels [mM] (fit) values [1/s] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) 10<sup>3</sup> values [mM] (fit) values [1/s] (fit) 10 <sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 10 gt + `å 10 g

Figure 14: Same as Figure 13, with noisy kinetic data

10 <sup>1</sup>

10 <sup>0</sup>

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

10 <sup>-3</sup>

Enzyme levels [mM] (true)

10<sup>-4</sup>

10 <sup>-2</sup>

10 <sup>1</sup>

10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup>

k<sub>cat</sub> values [1/s] (true)

10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup>

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

10<sup>-3</sup> 10<sup>-2</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup>

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

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

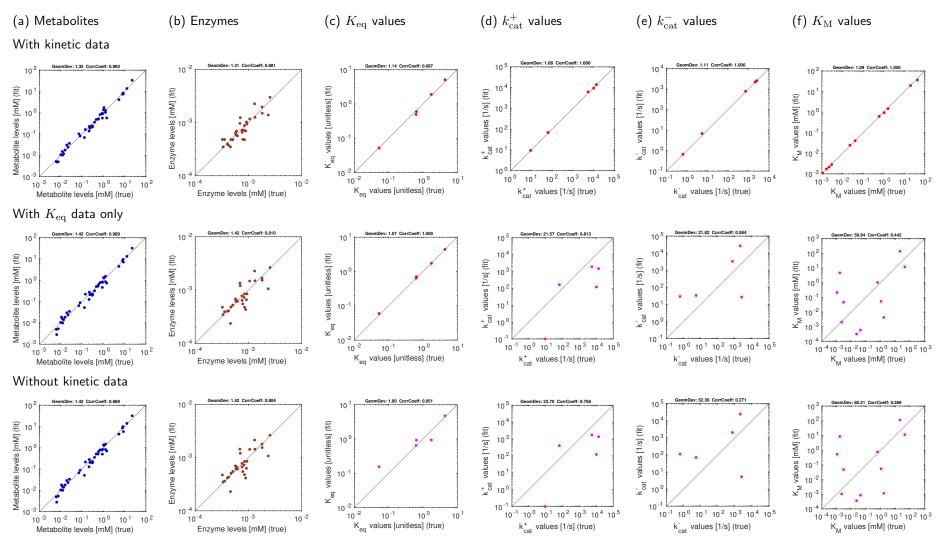


Figure 15: Results for Double branch with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial data, no data for kinetic constants).

Metabolite levels [mM] (true)

Enzyme levels [mM] (true)

#### Double branch model with artificial data (noisy kinetic data, noisy state data) - alpha = 1, beta = 0(d) $k_{\rm cat}^+$ values (a) Metabolites (b) Enzymes (c) $K_{\rm eq}$ values (e) $k_{\rm cat}^-$ values (f) $K_{ m M}$ values With kinetic data GeomDev: 1.48 CorrCoeff: 0.995 10 <sup>4</sup> 10 10 values [1/s] (fit) values [unitless] (fit) Metabolite levels [mM] (fit) 103 Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>0</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>0</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 + tg 10 , tg 10 ℃ . ≥ 10 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>1</sup> 10<sup>-1</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>-2</sup> 10<sup>0</sup> 10 <sup>1</sup> 10 -3 10 -2 10 -1 10 0 10 1 10-3 10-2 10-1 100 101 K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) k+ values [1/s] (true) K<sub>M</sub> values [mM] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) With $K_{\rm eq}$ data only GeomDev: 50.36 CorrCoeff: -0.108 GeomDev: 1.41 CorrCoeff: 0.814 GeomDev: 1.50 CorrCoeff: 0.964 GeomDev: 18.09 CorrCoeff: 0.832 10 10 4 10<sup>2</sup> 10 values [unitless] (fit) 10<sup>3</sup> Enzyme levels [mM] (fit) values [mM] (fit) 10 1 10 1 10 -2 values [1/s] (fit) values [1/s] (fit) 10<sup>3</sup> 10<sup>2</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 <sup>1</sup> 10<sup>-1</sup> Metabolite I , tg 10 ° , cat 10 0 10 1 10 2 10 3 10 4 10 5 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>0</sup> 10<sup>-1</sup> 10 <sup>1</sup> 10 -4 10 -3 10 -2 10 -1 10 0 10 1 10 2 10 3 10 -3 10 -2 10 -1 10 0 10 1 10<sup>-4</sup> 10<sup>-3</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 GeomDev: 23.70 CorrCoeff: 0.758 10<sup>5</sup> GeomDev: 85.21 CorrCoeff: 0.369 10<sup>-2</sup> 10 10 10 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 1 10 -1 10 -2 values [1/s] (fit) 10<sup>0</sup> 10<sup>3</sup> 10<sup>3</sup> 10<sup>2</sup> 10<sup>2</sup> 10 <sup>1</sup> 10 <sup>1</sup> 10<sup>-1</sup> 10 و علم مح cat 10<sup>0</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>3</sup> 10<sup>4</sup> 10<sup>5</sup> 10<sup>-1</sup> 10<sup>0</sup> 10 <sup>1</sup> 10 $^{-4}$ 10 $^{-3}$ 10 $^{-2}$ 10 $^{-1}$ 10 $^{0}$ 10 $^{1}$ 10 $^{2}$ 10 $^{3}$ 10 -3 10 -2 10 -1 10 0 10 1 K<sub>eq</sub> values [unitless] (true) k<sub>cat</sub> values [1/s] (true)

Figure 16: Same as Figure 15, with noisy kinetic data

k+ values [1/s] (true)

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

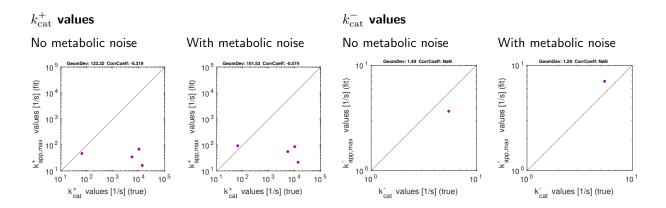


Figure 17: Catalytic constants in Double branch (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).