- 1 Three-chain model Simulations with alpha = 0.1
- 2 Three-chain point model Simulations with alpha = 0.1, beta = 0

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

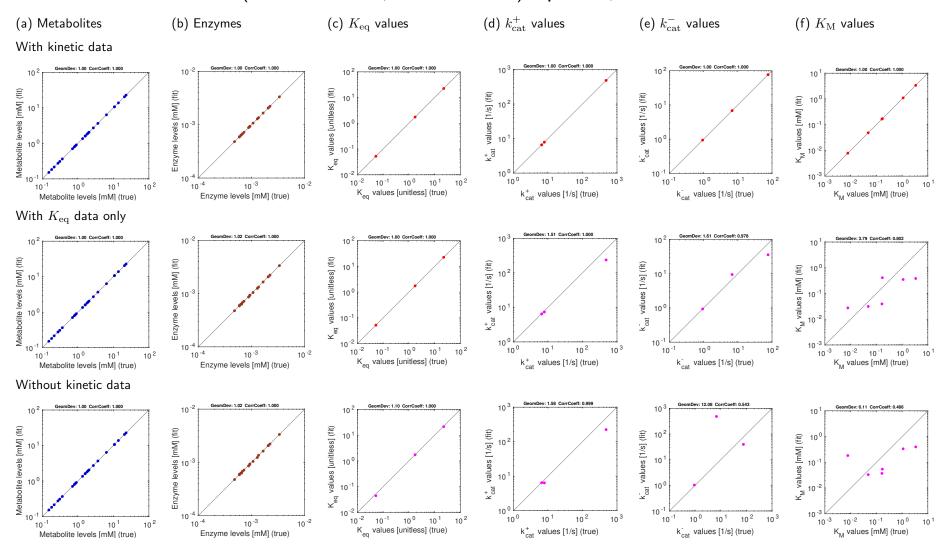


Figure 3: Model balancing results for Three-chain model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation coefficients ("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).

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

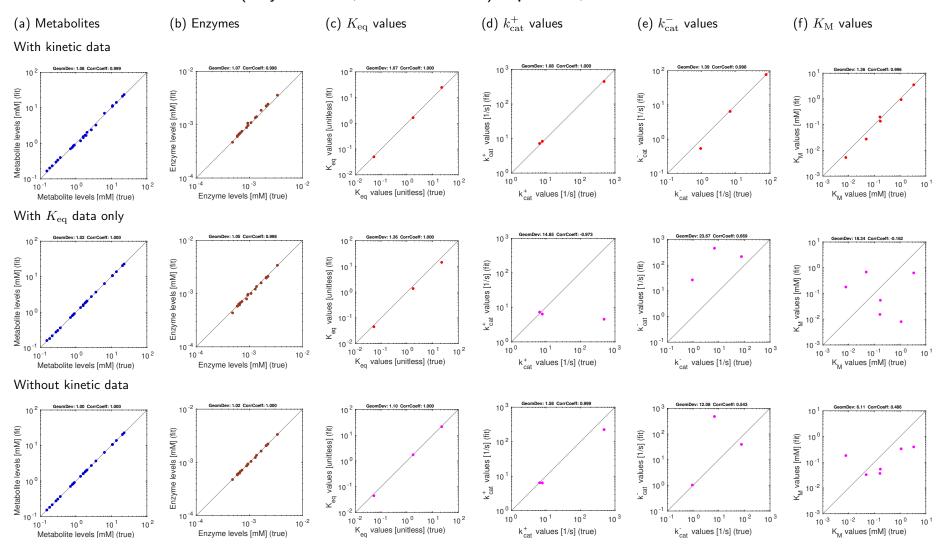


Figure 4: Same as Figure 8, with noisy kinetic data

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

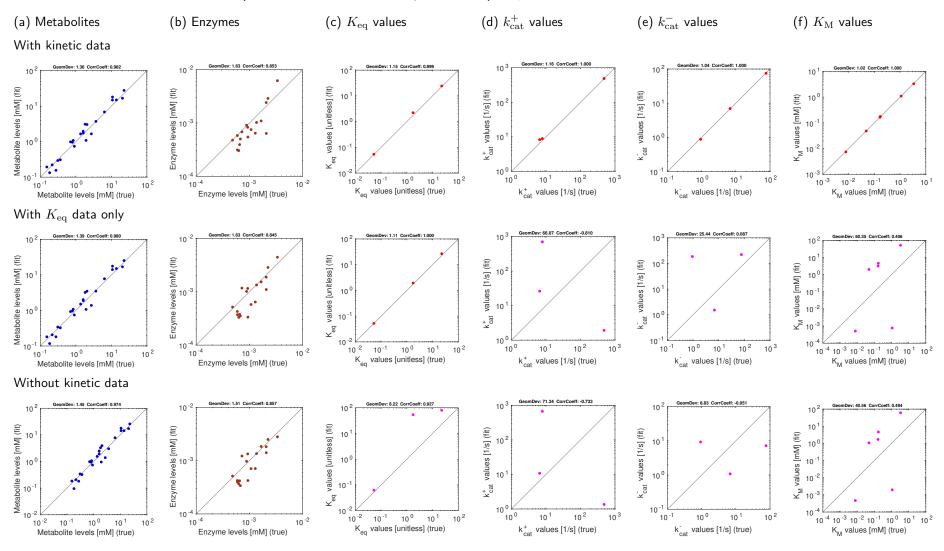


Figure 5: Results for Three-chain with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial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

#### Three-chain model with artificial data (noisy kinetic data, noisy state data) - alpha = 0.1, 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: 1.34 CorrCoeff: 0.998 GeomDev: 1.19 CorrCoeff: 0.999 10 102 GeomDev: 1.47 CorrCoeff: 0.989 10<sup>2</sup> 10<sup>2</sup> 10 Metabolite levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) Enzyme levels [mM] (fit) 10 values [1/s] (fit) values [mM] (fit) 10<sup>1</sup> 10<sup>0</sup> 10 -10<sup>0</sup> 10 + cat , cat ⊼<sub>@</sub> 10<sup>2</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>3</sup> 10<sup>1</sup> 10<sup>2</sup> 10<sup>-2</sup> 10 <sup>1</sup> 10 <sup>-1</sup> 10 <sup>-3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> K<sub>eq</sub> values [unitless] (true) k<sub>cat</sub> 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 $K_{\mathrm{eq}}$ data only GeomDev: 17.90 CorrCoeff: 0.163 10 10<sup>3</sup> GeomDev: 54.66 CorrCoeff: 0.406 10-2 10 Metabolite levels [mM] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) 10 10 values [1/s] (fit) 10<sup>2</sup> values [mM] (fit) 10<sup>0</sup> 10 <sup>1</sup> 10<sup>0</sup> 10 -10 -2 10<sup>0</sup> 10 k+ cat , cat ⊼ 8 10 " 10<sup>2</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>3</sup> 10<sup>-1</sup> 10<sup>3</sup> 10<sup>-2</sup> 10<sup>-1</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 <sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> 10 <sup>-2</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: 1.51 CorrCoeff: 0.857 GeomDev: 8.22 CorrCoeff: 0.927 GeomDev: 8.83 CorrCoeff: -0.051 GeomDev: 40.56 CorrCoeff: 0.494 102 102 10 -2 10 Metabolite levels [mM] (fit) values [1/s] (fit) 10 values [unitless] (fit) Enzyme levels [mM] (fit) values [1/s] (fit) values [mM] (fit) 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10 <sup>0</sup> 10<sup>0</sup> 10<sup>-1</sup> , cat κ cat 10 -10<sup>2</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>3</sup> 10<sup>-1</sup> 10 <sup>0</sup> 10 <sup>1</sup> 10<sup>2</sup> 10 <sup>0</sup> 10 <sup>1</sup> 10 -4 10 -3 10 -2 10 -1 10 0 10 1 10 2 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>1</sup> 10<sup>2</sup> K<sub>eq</sub> values [unitless] (true)

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

Enzyme levels [mM] (true)

Metabolite levels [mM] (true)

k+ values [1/s] (true)

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

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

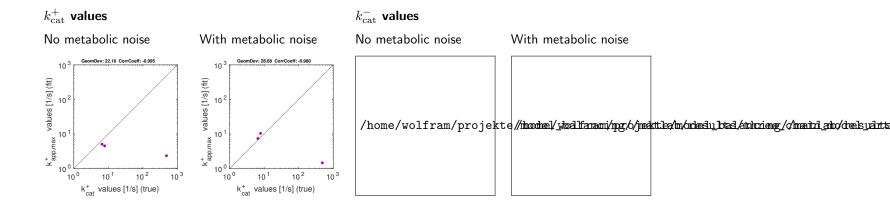


Figure 7: Catalytic constants in Three-chain (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\text{cat}}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\text{cat}}^+$  for reactions with forward fluxe).

- 3 Three-chain model Simulations with alpha = 1
- 4 Three-chain point model Simulations with alpha = 1, beta = 0

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

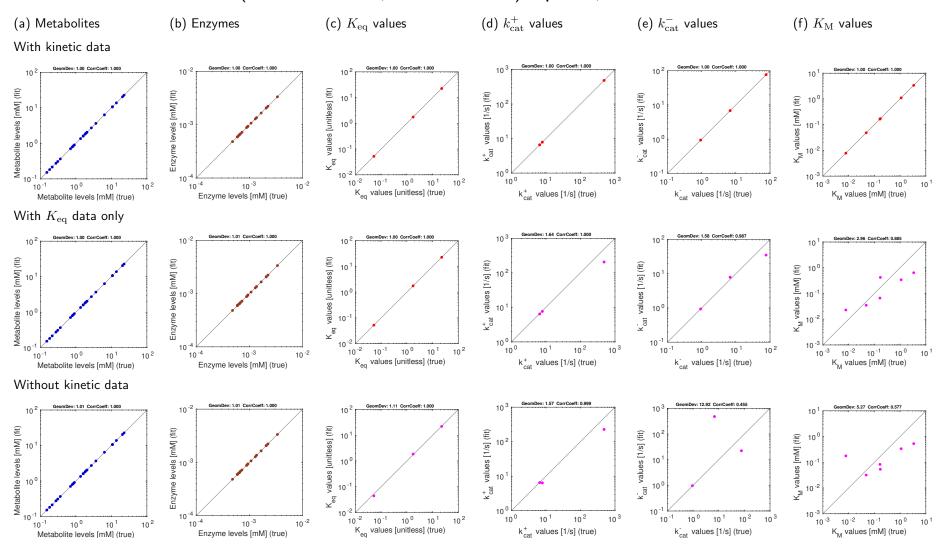


Figure 8: Model balancing results for Three-chain model with artificial data. The model structure is shown in Figure ??. Each subfigure shows "true" values (x-axis) versus reconstructed values (y-axis). Similarities are quntified by geometric standard deviations ("GeomDev") and Pearson correlation coefficients ("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).

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

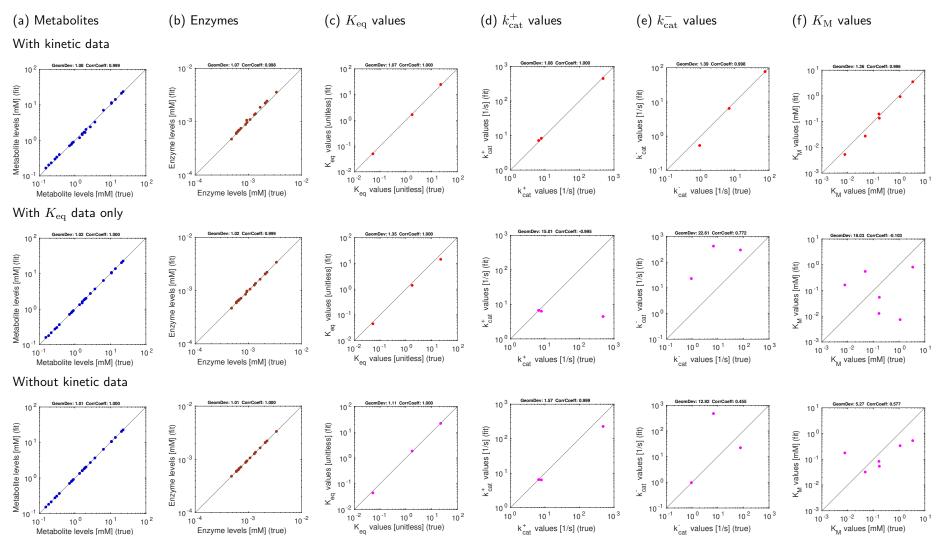


Figure 9: Same as Figure 8, with noisy kinetic data

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

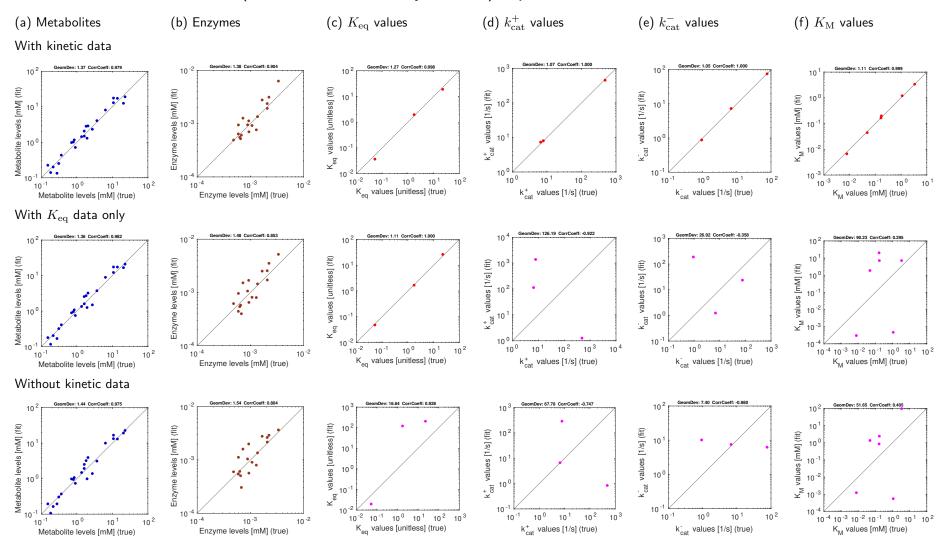


Figure 10: Results for Three-chain with noisy artificial data. Top row: estimation scenario S3 (noisy artificial data, data used for kinetic constants). Centre row: estimation scenario S3K (noisy artificial data, data for equilibrium constants only). Bottom row: estimation scenario S4 (noisy artificial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

10<sup>-1</sup>

10 <sup>1</sup>

Metabolite levels [mM] (true)

10<sup>2</sup>

#### Three-chain 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: 1.23 CorrCoeff: 0.999 GeomDev: 1.20 CorrCoeff: 0.999 10 102 GeomBey: 1.49 CorrCoeff: 0.986 10<sup>2</sup> 10<sup>2</sup> Metabolite levels [mM] (fit) values [unitless] (fit) values [1/s] (fit) Enzyme levels [mM] (fit) 10 values [1/s] (fit) values [mM] (fit) 10<sup>1</sup> 10<sup>0</sup> 10 -10<sup>0</sup> 10 + cat , cat ⊼<sub>@</sub> 10<sup>2</sup> 10<sup>0</sup> 10<sup>1</sup> 10<sup>3</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-2</sup> 10<sup>0</sup> 10 <sup>1</sup> 10 <sup>-1</sup> 10 <sup>-3</sup> 10 <sup>-2</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> K<sub>eq</sub> values [unitless] (true) k<sub>cat</sub> values [1/s] (true) K<sub>M</sub> values [mM] (true) k+ values [1/s] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) With $K_{\mathrm{eq}}$ data only GeomDev: 84.67 CorrCoeff: -0.747 GeomDev: 18.49 CorrCoeff: 0.019 10 10<sup>3</sup> GeomDev: 62.84 CorrCoeff: 0.366 10-2 10 vels [mM] (fit) values [1/s] (fit) Enzyme levels [mM] (fit) values [unitless] (fit) 10 10 values [1/s] (fit) 10<sup>2</sup> values [mM] (fit) 10<sup>0</sup> 10 <sup>1</sup> 10<sup>0</sup> 10 Metabolite lev 10 -2 10<sup>0</sup> 10 k+ cat , cat ⊼ 8 10<sup>2</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>1</sup> 10<sup>3</sup> 10<sup>-1</sup> 10<sup>3</sup> 10<sup>-2</sup> 10<sup>-1</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<sup>0</sup> 10 <sup>1</sup> 10<sup>2</sup> 10<sup>-4</sup> 10 <sup>-2</sup> K<sub>eq</sub> values [unitless] (true) k at values [1/s] (true) K<sub>M</sub> values [mM] (true) k<sub>cat</sub> values [1/s] (true) Metabolite levels [mM] (true) Enzyme levels [mM] (true) Without kinetic data nDev: 1.54 CorrCoeff: 0.804 GeomDev: 67.78 CorrCoeff: -0.747 GeomDev: 51.65 CorrCoeff: 0.405 10<sup>2</sup> 10-2 10 10<sup>2</sup> Metabolite levels [mM] (fit) 10<sup>1</sup> values [unitless] (fit) Enzyme levels [mM] (fit) 10<sup>2</sup> values [mM] (fit) values [1/s] (fit) values [1/s] (fit) 10<sup>2</sup> 10 <sup>1</sup> 10 10 <sup>1</sup> 10<sup>0</sup> 10<sup>0</sup> te 10 0 } k cat 10

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

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

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

10<sup>-4</sup>

10 <sup>-3</sup>

Enzyme levels [mM] (true)

10 <sup>-2</sup>

10<sup>2</sup>

10<sup>0</sup> 10<sup>1</sup>

k+ values [1/s] (true)

10 <sup>-1</sup>

10 <sup>1</sup>

k cat values [1/s] (true)

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>

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

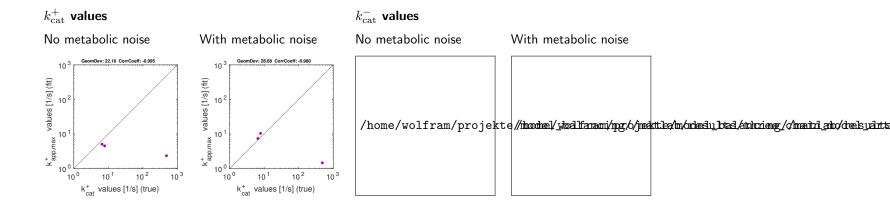


Figure 12: Catalytic constants in Three-chain (artificial data), estimated by kinetic profiling [?]. Note that  $k_{\rm cat}$  values can only be estimated in the direction of fluxes (e.g.  $k_{\rm cat}^+$  for reactions with forward fluxe).