Appendix

Systematic mapping of protein-metabolite interactions in central metabolism of Escherichia coli.
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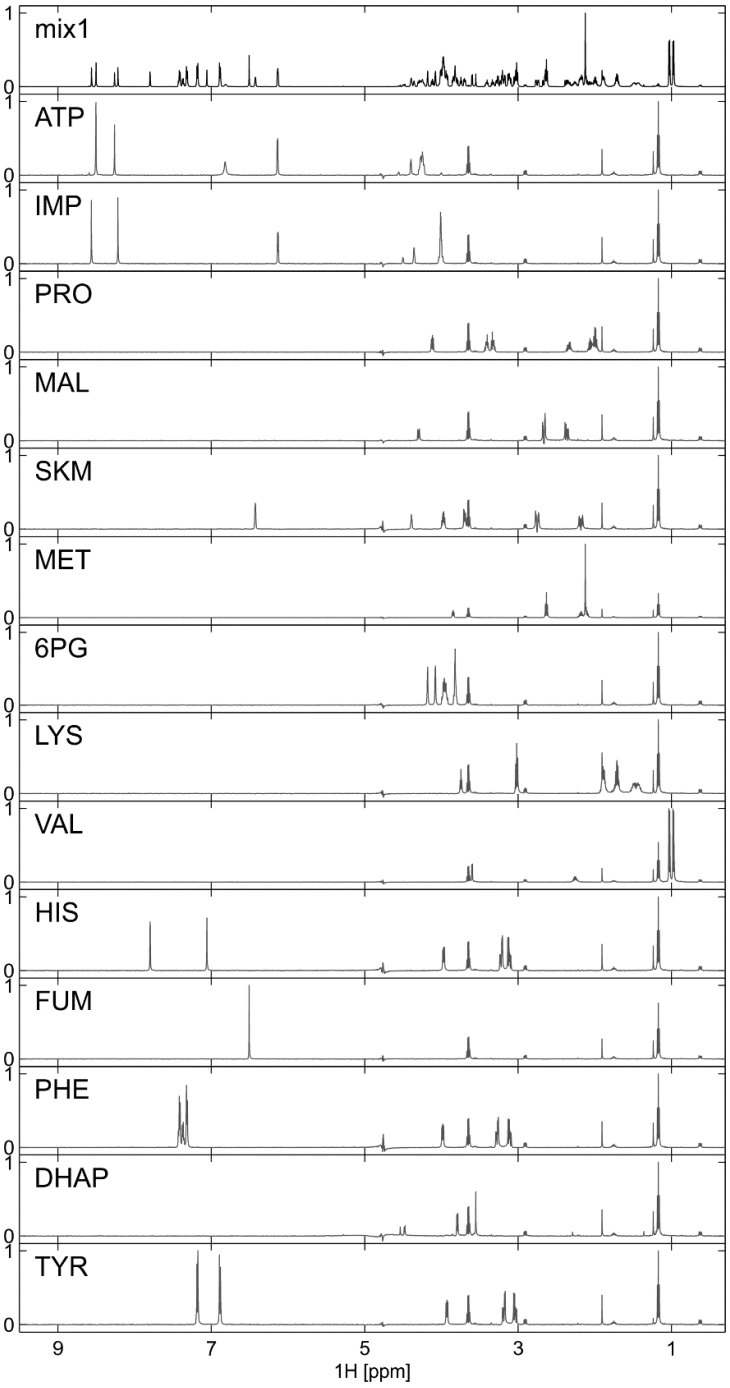
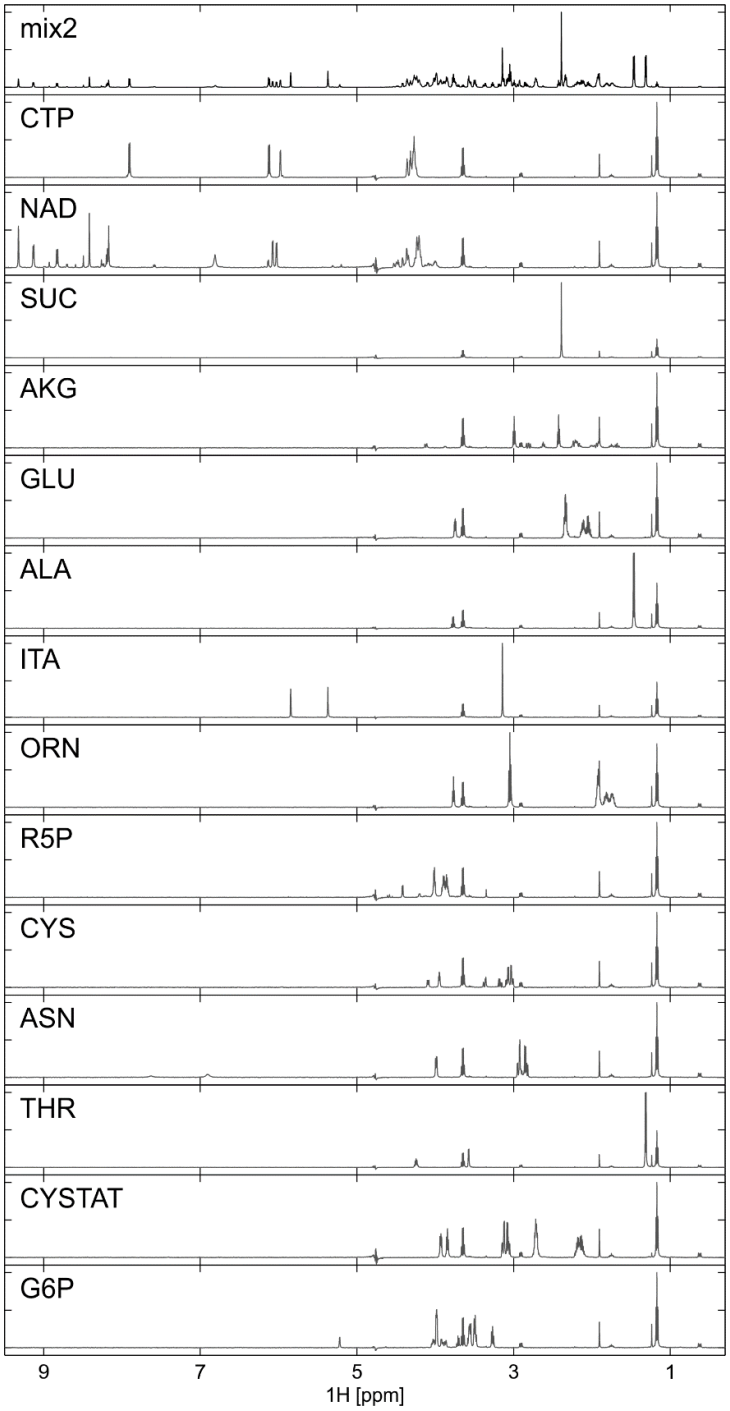
Table of Contents



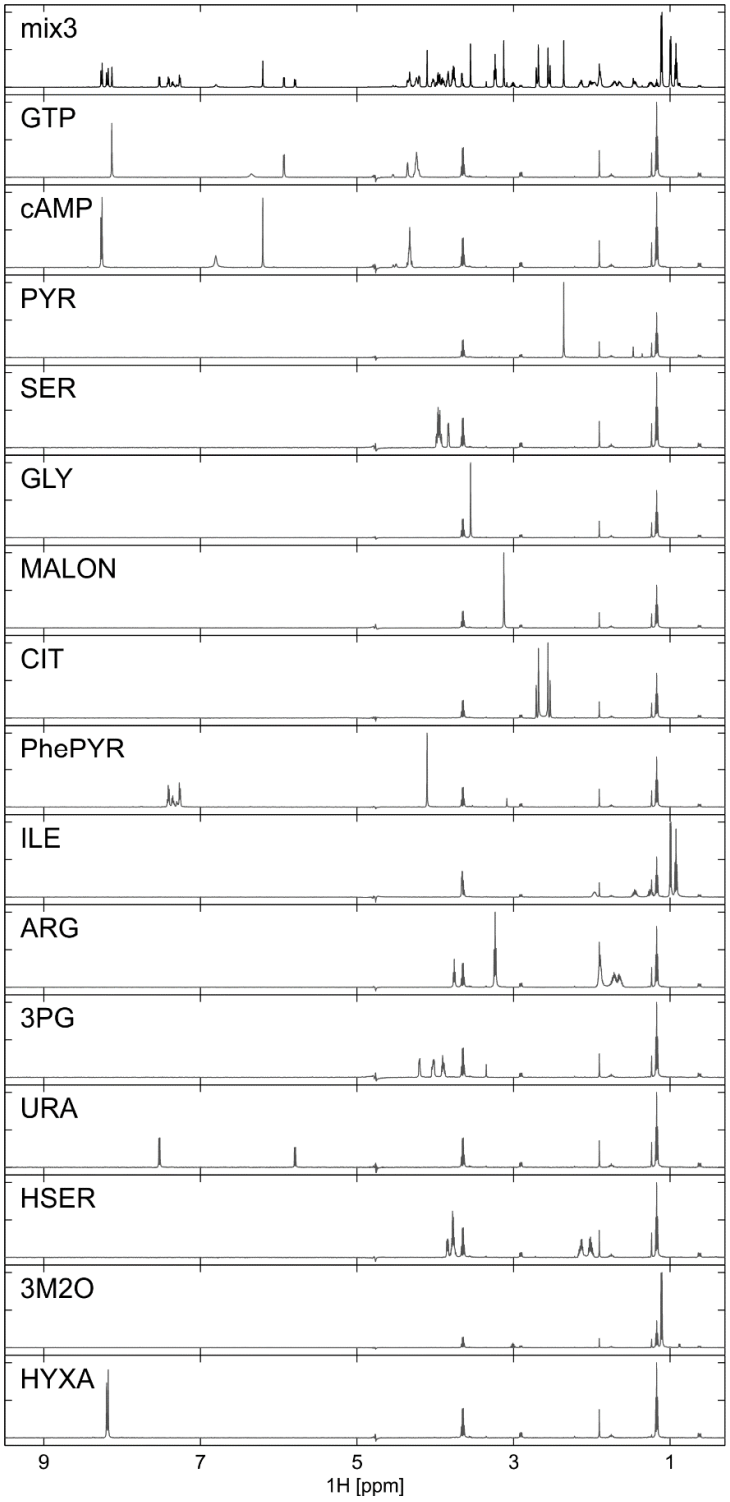
Appendix Figure S1: Reference spectra for metabolite mix 1.

1D ^{1}H-NMR spectra of metabolite mix 1 and the individual metabolites contained therein.



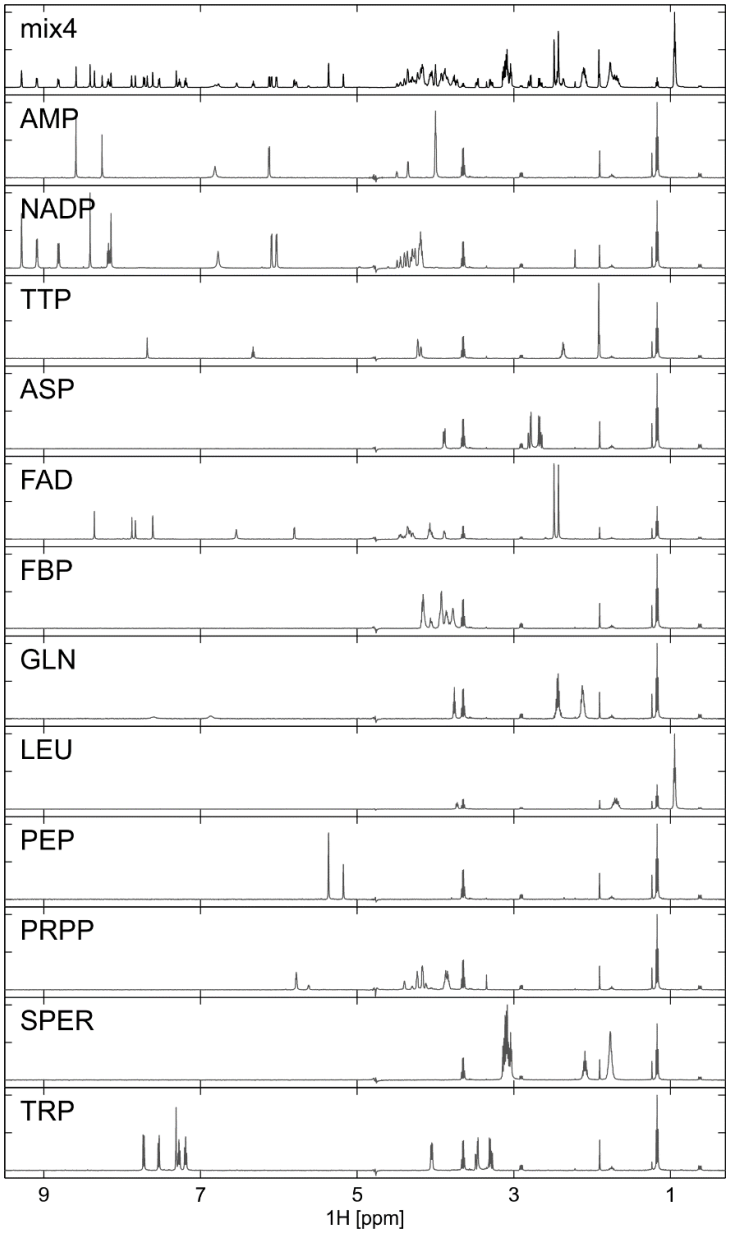
Appendix Figure S2: Reference spectra for metabolite mix 2.

1D ^{1}H-NMR spectra of metabolite mix 2 and the individual metabolites contained therein.



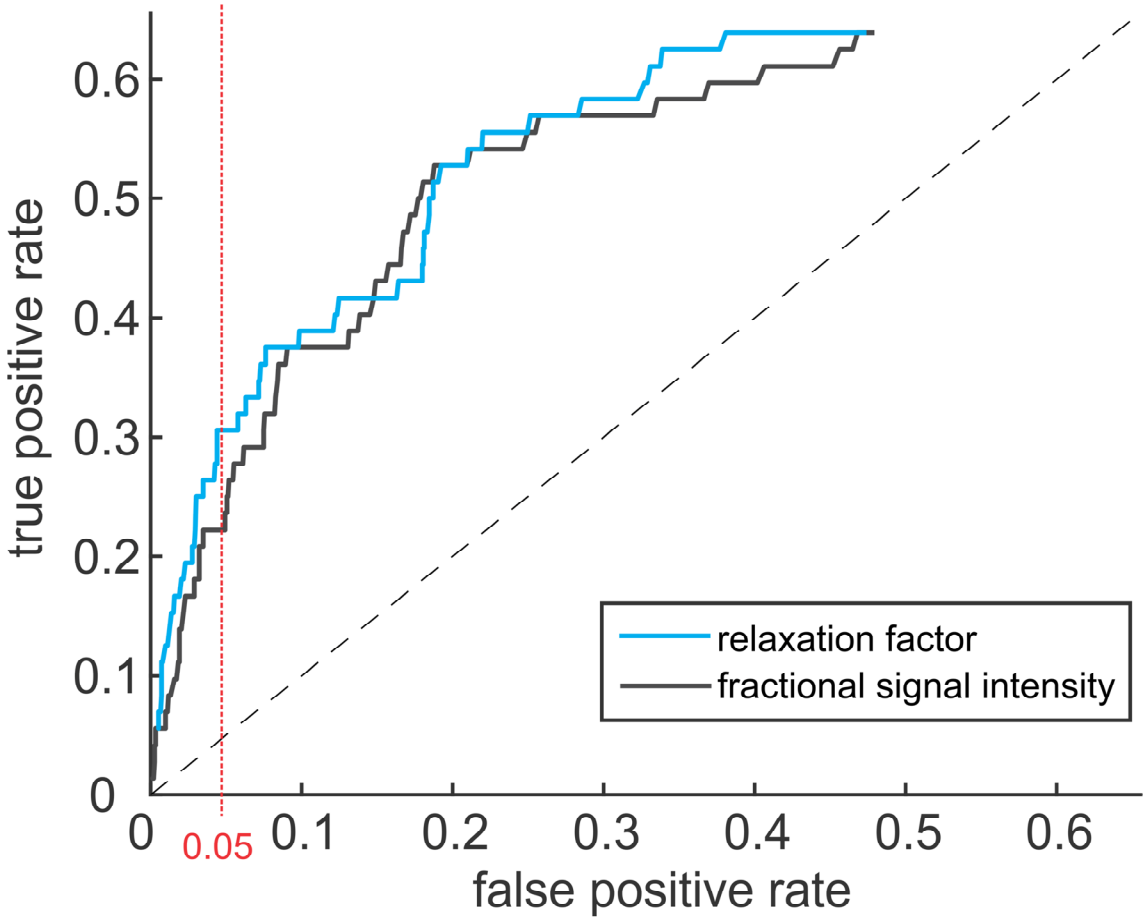
Appendix Figure S3: Reference spectra for metabolite mix 3.

1D ^{1}H-NMR spectra of metabolite mix 3 and the individual metabolites contained therein.



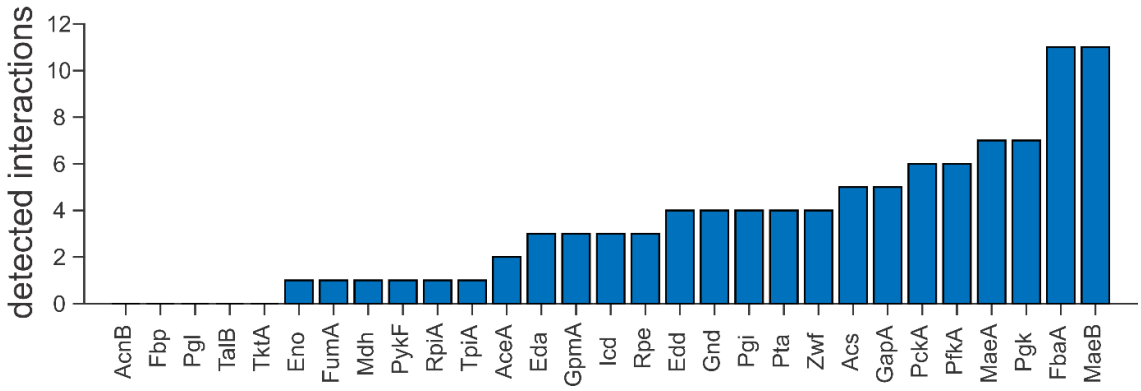
Appendix Figure S1: Reference spectra for metabolite mix 4.

1D ^{1}H-NMR spectra of metabolite mix 4 and the individual metabolites contained therein.



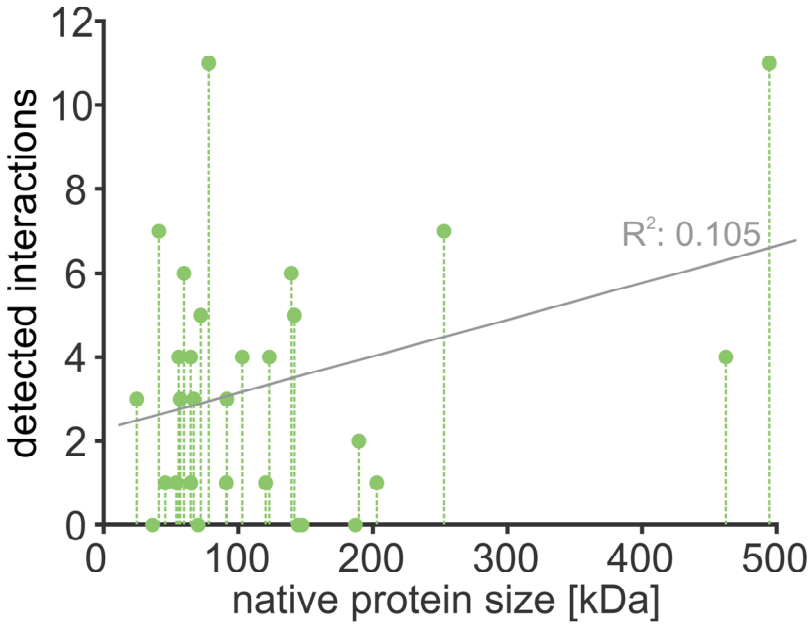
Appendix Figure S5: Receiver-operator characteristics curve for the recovery of known interactions.

The false-positive and true-positive rates of recovering known interactions from EcoCyc were calculated for different cutoffs of the relaxation factor ΔRF (blue line) and the fractional signal intensity (black line).



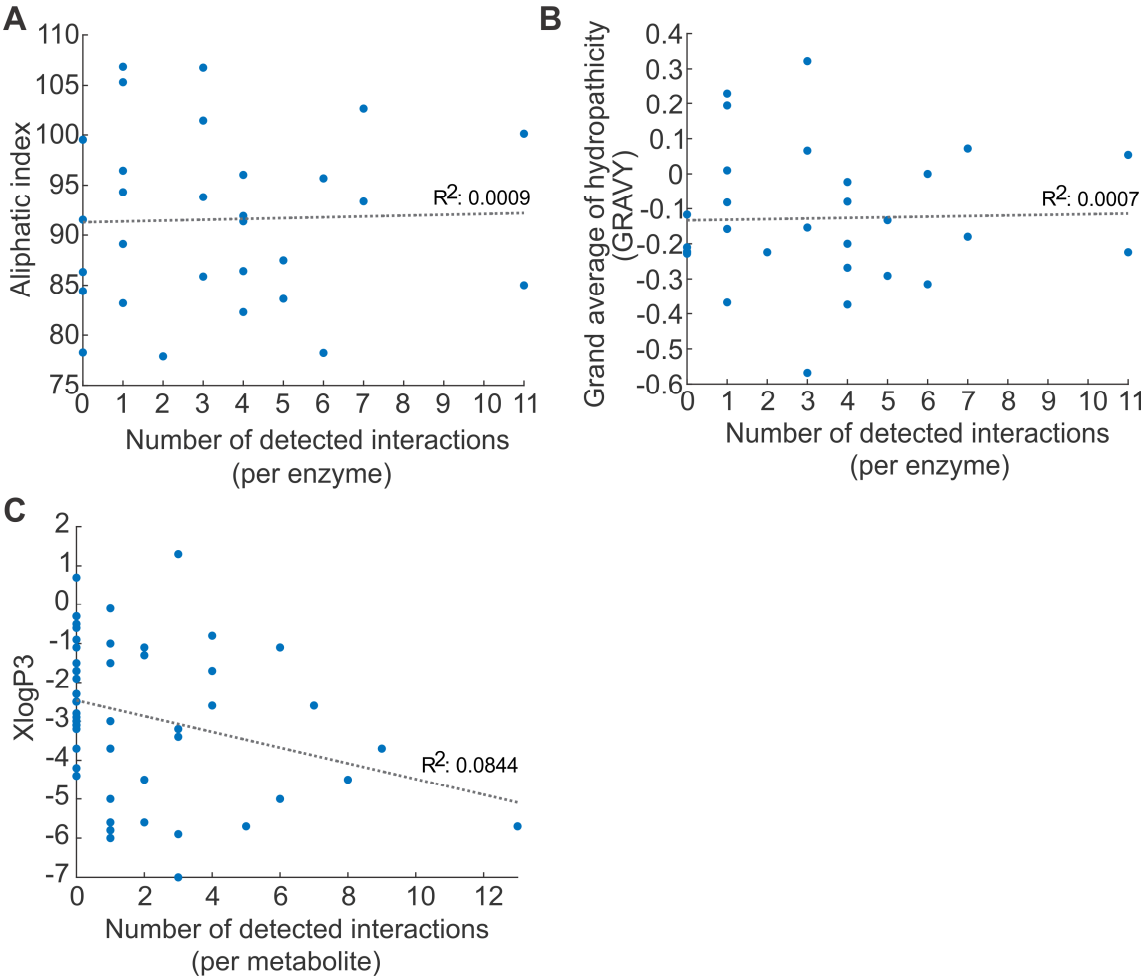
Appendix Figure S6: Number of detected interactions per enzyme.

The height of the bars indicate the number of interactions detected with NMR.



Appendix Figure S7: Number of detected interactions per enzyme versus native protein size.

The detected interactions per protein (ΔRF > 0.1805) are plotted against the native protein size. The grey line represents a linear fit with R^2 = 0.105.

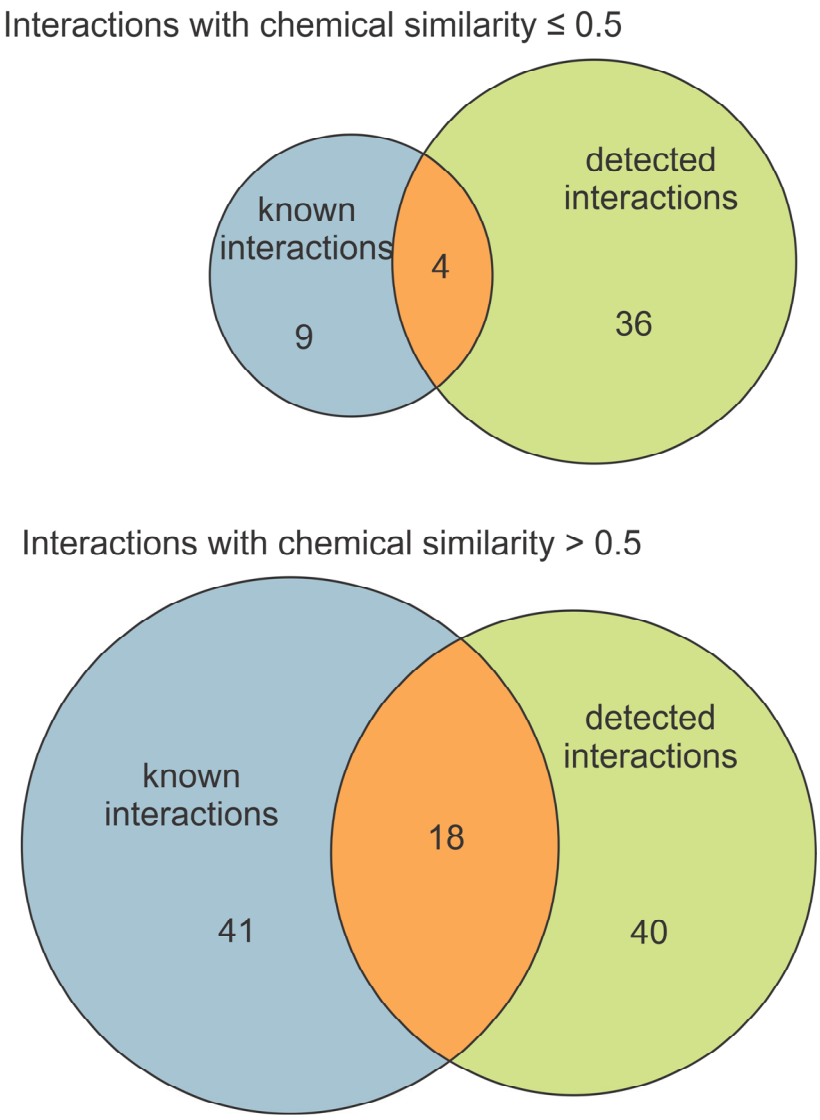


Appendix Figure S8: Correlation of protein and metabolite hydrophobicity with number of detected interactions.

A, Correlation of protein aliphatic index (from sequence level analysis) with the number of detected interactions per protein. The grey dotted line represents a linear fit with R^2 = 0.0009.

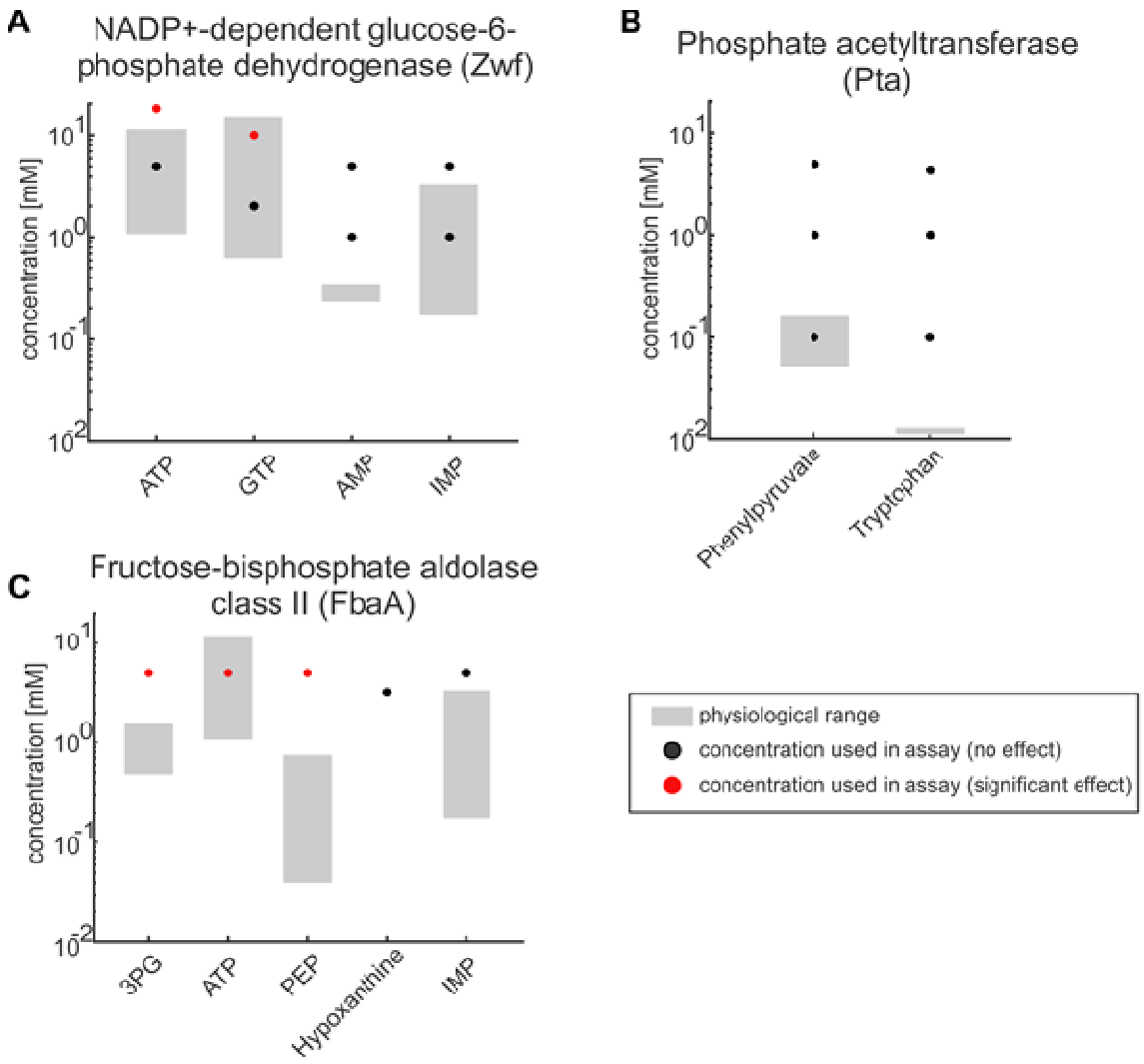
B, Correlation of protein grand average of hydropathicity (from sequence level analysis) with the number of detected interactions per protein. The grey dotted line represents a linear fit with R^2 = 0.0007.

C, Correlation of metabolite lipophilicity (XLogP3, from PubChem) with the number of detected interactions per metabolite. The grey dotted line represents a linear fit with R^2 = 0.0844.



Appendix Figure S9: Chemical similarity of new and known interactions.

For metabolite enzyme-interactions with a score above 0.5 and below 0.5 the number of known, known and detected and newly detected interactions is presented in blue, orange and green respectively.



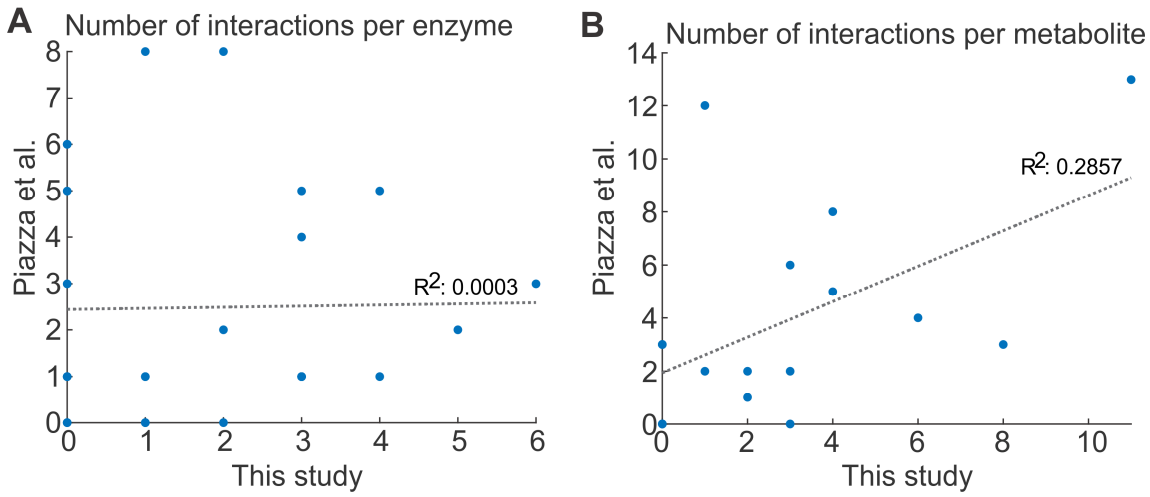
Appendix Figure S10: Comparison between *in vivo* and *in vitro* metabolite concentrations.

The grey bar denotes the *in vivo* steady state concentrations of all metabolites used in *in vitro* enzyme assays. Data was extracted from Park et al, 2016 and Kochanowski et al, 2017, for hypoxanthine, no *in vivo* concentration was available.

A, *In vivo* and *in vitro* metabolite concentrations for enzyme assay with NADP+-dependent glucose-6-phosphate dehydrogenase.

B, *In vivo* and *in vitro* metabolite concentrations for enzyme assay with phosphate acetyltransferase.

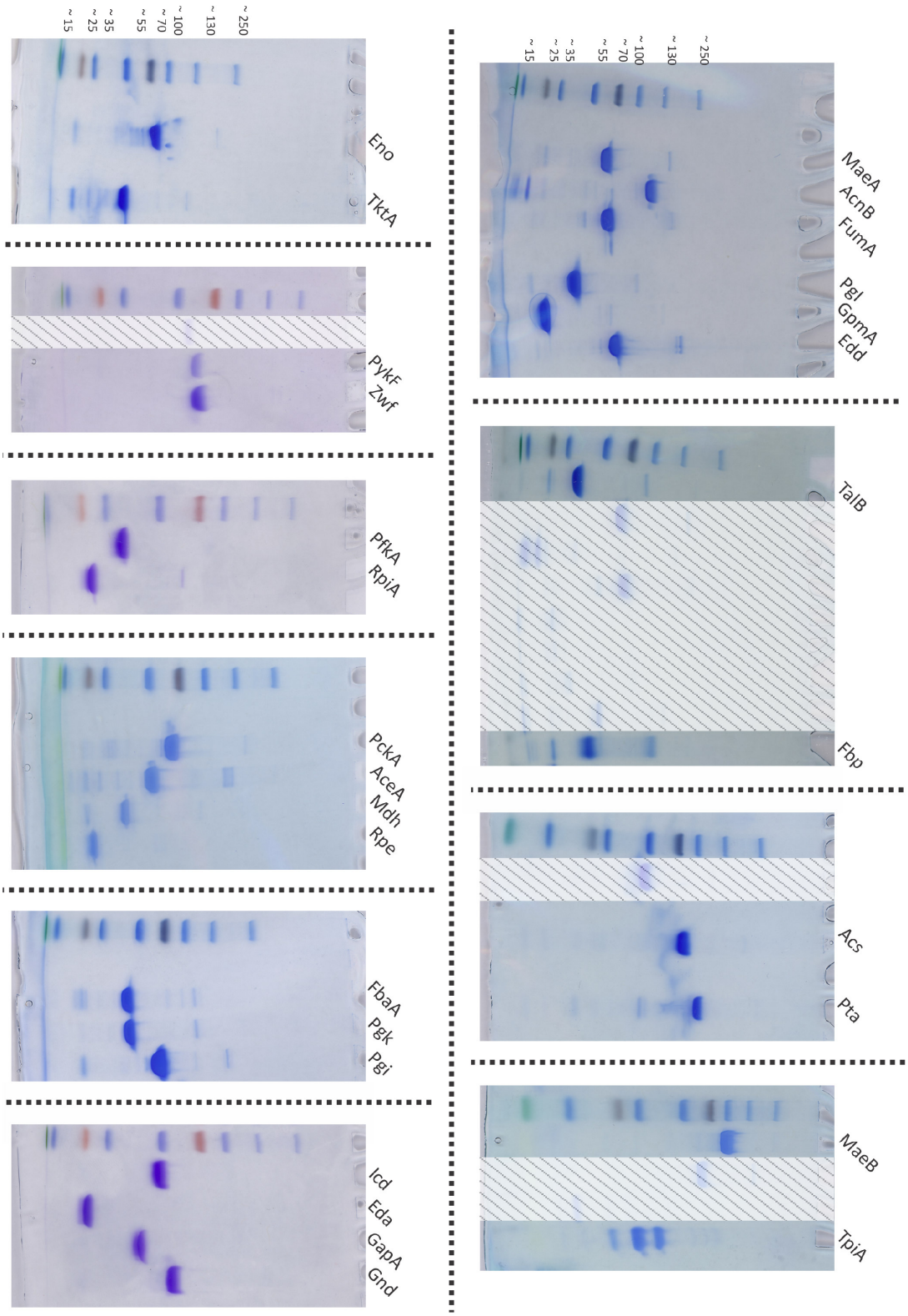
C, In vivo and in vitro metabolite concentrations for enzyme assay with fructose-bisphosphate aldolase class II.



Appendix Figure S11: Correlation of interactions detected per enzyme and metabolite in this study and Piazza et *al.* 2018.

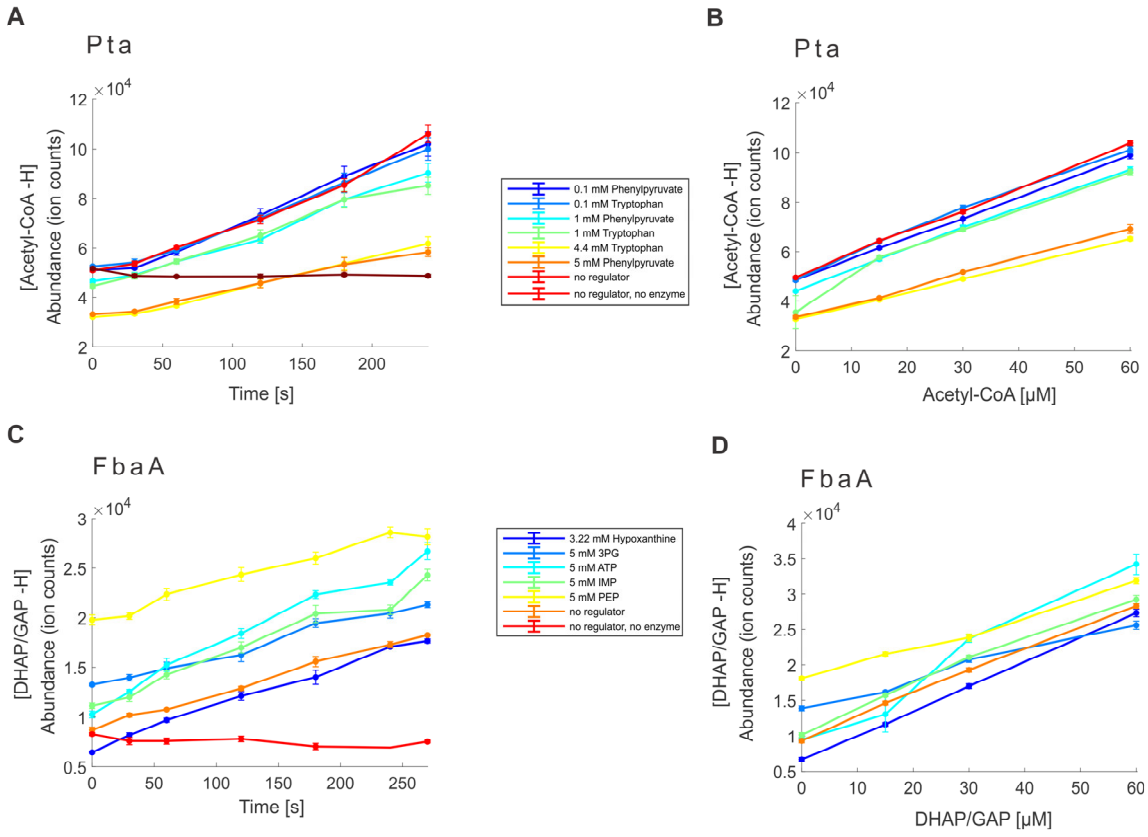
A, Correlation of the number of interactions detected per protein. The grey dotted line represents a linear fit with $R^2 = 0.0003$.

B, Correlation of the number of interactions detected per metabolite. The grey dotted line represents a linear fit with $R^2 = 0.2857$.



Appendix Figure S12: SDS-PAGE of all tested proteins.

The approximate marker sizes are indicated in kDa (Precision Plus Protein Dual Color standard, Bio-Rad).



Appendix Figure S13: Raw data for enzyme assays with Pta and FbaA.

A, Increase of acetyl-CoA (product of Pta) over time, n= 3.

B, Standard curve for acetyl-CoA in the presence of different amounts of inhibitors (without added enzyme).

C, Increase of dihydroxyacetone phosphate/ glyceraldehyde-3-phosphate (products of FbaA) over time, n= 3.

D, Standard curve for dihydroxyacetone phosphate/ glyceraldehyde-3-phosphate in the presence of different amounts of inhibitors (without added enzyme).