molecular systems biology

Systematic mapping of protein-metabolite interactions in central metabolism of *Escherichia coli*

Maren Diether, Yaroslav Nikolaev, Frédéric H.T. Allain and Uwe Sauer.

Review timeline:

Submission date:	17th May 2019
Editorial Decision:	28th June 2019
Revision received:	18th July 2019
Accepted:	31st July 2019

Editor: Maria Polychronidou

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 28th June 2019

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your study. As you will see below, the reviewers raise a series of concerns, which we would ask you to address in a revision.

Reviewers #1 and #2 are quite supportive and raise only a few issues that I think there is no need to repeat here. Reviewer #2, who would not be opposed to revealing their identity to you, has asked us to forward you a PDF with comments made directly on the manuscript text (attached below). These few comments on how to improve the study refer to clarifications and very minor text changes. Reviewer #3 is somewhat less supportive and raises concerns regarding the physiological and in vivo relevance of the reported interactions. We would ask you to clarify this issue in the revised manuscript. Please feel free to contact me in case you would like to discuss in further detail any of the issues raised.

REFEREE REPORT

Reviewer #1:

The authors present their application of ligand detected NMR to the enzymes of central carbon metabolism in E.coli. Using this established approach, they identify candidate allosteric regulators of several of these enzymes and validate a few interactions in vitro.

The addition of two pieces of information would help to place these results in biological context.

First, the authors describe a successive data acquisition used to rule out "unstable" compounds. Are some of these in fact the result of catalysis? If so, this bears further discussion in the text and

perhaps in the supplementary data as well, as it provides potential guidance about the substrate specificity. (Of course, contaminating avtivitres may be an issue, but this can be discussed.)

In addition, the potential regulators can be put into better context by also providing the literature values for metabolite concentration. The concentrations tested here are in typical physiological range for some compounds, but well above for others. While an effect at high concentration may well be relevant in a specific physiological context, including the typical concentrations of these regulators and potential regulators will help the audience put them in context for their relevance to typical conditions.

Reviewer #2:

Regulation of enzymes via metabolites is a critical layer of cell regulation. Yet, due to limited technical capabilities it is a notoriously limited in its characterisation.
With that situation as background, new techniques are urgently needed, and the current paper is the most impressive exploration us such an approach I saw. I view this paper as highly deserving of publication. I wrote specific comments on the manuscript which I was hoping to communicate to the authors.

I do not have an objection that my identity will be revealed via the commented manuscript

Reviewer #3:

The authors studied metabolite protein interactions in the e.coli central metabolism. Therefore 29 proteins were expressed and purified and tested with 4 mixtures containing in total 55 metabolites.
The protein-metabolite interactions were analyzed using NMR based methods.
The authors report that they found 98 interactions with 76 being novel.
However, only 30% of the known interactions could be verified and only 5 out of 8 novel interactions that the authors scored as allostearic were shown regulatory.
Furthermore the functionality of these interactions was found at extreme high concentrations of the interacting metabolites (e.g. 10mM).
Do the authors see the opportunity that such high concentrations can be achieved in vivo?
Why could the known interactions not be retrieved?

In this stage the study is very preliminary and does not lead to novel insights or regulatory principles, yet. The regulatory interactions seem at extreme concentrations, thus the relevance upon 'normal' metabolic regulation may be questioned.
If one would calculate the success of the method to measure the known interactions and the frequency that identified allostearic binders influence the activity of the target enzyme at extreme conditions the the publication is not mature at this stage.

1st Revision - authors' response 18th July 2019

Reviewer #1:

The authors present their application of ligand detected NMR to the enzymes of central carbon metabolism in E.coli. Using this established approach, they identify candidate allosteric regulators of several of these enzymes and validate a few interactions in vitro.

The addition of two pieces of information would help to place these results in biological context.

First, the authors describe a successive data acquisition used to rule out "unstable" compounds. Are some of these in fact the result of catalysis? If so, this bears further discussion in the text and perhaps in the supplementary data as well, as it provides potential guidance about the substrate specificity. (Of course, contaminating activities may be an issue, but this can be discussed.)

It is indeed conceivable that some of the compounds ruled out as 'unstable' were subject to catalysis by proteins. However, because protein-metabolite mixtures were incubated at room temperature for ~3-8 h before the actual NMR measurement, we assume that any significant catalytic conversions should already take place before the NMR measurements and thus, would not be observable during successive experimental acquisitions. Differentiating catalytic activity from other sources of compound instability is thus not easily possible in our current set up. Nevertheless, the data on such potential "catalytic" events is preserved in our datasets. We added a statement addressing this point in the first 'Results' paragraph:

"Among other sources of instability, metabolite degradation could be the result of enzymatic conversion, although this is not likely to be a major confounding factor given that the protein metabolite mixture was incubated for several hours prior to NMR recording. However, differentiating the various sources of metabolite instability is not feasible given our current setup. "

In addition, the potential regulators can be put into better context by also providing the literature values for metabolite concentration. The concentrations tested here are in typical physiological range for some compounds, but well above for others. While an effect at high concentration may well be relevant in a specific physiological context, including the typical concentrations of these regulators and potential regulators will help the audience put them in context for their relevance to typical conditions.

To help readers to put our results into the context of typical cellular conditions we added an Appendix figure (S10) comparing physiological metabolite concentrations (from Park 2016 and Kochanowski 2017) with the concentrations used in our *in vitro* enzyme assays. Note that the anticipated "physiological concentrations" were sampled from only a few experiments with narrow set of conditions each, and thus likely represent only a part of the full physiological concentration range. In summary, of the five interactions showing confident effect on enzyme activity, two metabolites show the effect within their physiological concentration range (Zwf-GTP, FbaA-ATP), and three more show the effect at concentration 1- to 7-fold higher than the anticipated physiological concentration range of the metabolite (Zwf-ATP, FbaA-3PG, FbaA-PEP). We included a statement on physiological concentration ranges in the third paragraph in the 'Discussion' section.

In summary, of the five interactions showing significant effects on enzyme activity, two metabolites show the effect within their physiological concentration range (Zwf-GTP, FbaA-ATP), and three more show the effect at concentrations 1- to 7-fold higher than the anticipated physiological steady state concentration range of the metabolite (Zwf-ATP, FbaA-3PG, FbaA-PEP) (Park et al, 2016; Kochanowski et al, 2017) (Appendix Fig. S10).

More generally, the NMR experiments used in our study detect interactions with dissociation constants (K_Ds) in the μM-to-mM range (mentioned in the last paragraph of section 'Results, Ligand-detected T1rho NMR assay for a biological subnetwork').

Reviewer #2:

Regulation of enzymes via metabolites is a critical layer of cell regulation. Yet, due to limited technical capabilities it is a notoriously limited in its characterisation.

With that situation as background, new techniques are urgently needed, and the current paper is the most impressive exploration us such an approach I saw. I view this paper as highly deserving of publication. I wrote specific comments on the manuscript which I was hoping to communicate to the authors.

I do not have an objection that my identity will be revealed via the commented manuscript

Comments of reviewer 2 (extracted from pdf annotation)
Comments regarding the introduction
Sentence: One challenge is the generally low affinity of protein-metabolite interactions
Comment: It would be useful in my opinion if an order of magnitude was given. Say mM.

We adapted the sentence as follows: *One challenge is the generally low affinity (mM range) of protein-metabolite interactions (Reznik et al, 2017), and their fleeting nature.*

Sentence: At present about 100 regulatory and 130 catalytic interactions involving …
Comment: Would be useful to define regulatory versus catalytic interactions, maybe via an example.

We adapted the sentence as follows: *At present about 100 regulatory (metabolite changes enzyme activity) and 130 catalytic (metabolite is substrate or product) interactions involving the 35 major isoenzymes of central metabolism are reported in the EcoCyc database (Keseler et al, 2017).*

Sentence: … by choosing cutoffs that recovered 30% of all known interactions at a false-positive rate of 5%.
Comment: This sounds to be low, please motivate the choice

We rephrased the sentence to increase clarity: *Here, we focused our analysis only on high confidence NMR interactions by choosing a false-positive rate cutoff of 5%, which yielded a dataset encompassing 30% of the 72 known interactions.*

Sentence: At this cutoff, we detected 98 interactions between all tested enzymes and metabolites, including 76 interactions that had not been reported previously,
Comment: how many of the known interactions were recovered? 30% of the 100+130 mentioned above?

We included the following statement: *"We systematically generated ligand-detected NMR interaction profiles of 29 purified enzymes from E. coli central metabolism with 55 selected metabolites, between which 72 interactions were already known."*

Comments regarding the results section: Ligand-detected T1rho NMR assay for a biological subnetwork

Sentence: Additionally, we selected metabolites from branch points of metabolic pathways, based on the assumption that these are more likely to exert regulatory roles.
Comment: is there a ref to this?

Previous studies have shown that metabolic regulation is more likely to occur at branch point enzymes. However, preferential regulatory roles for branch point metabolites have not been demonstrated yet. We removed the corresponding sentence to avoid confusion.

Sentence: To detect protein-metabolite interactions, purified proteins were mixed with a subset of metabolites and NMR spectra were recorded.
Comment: Please explain the method in a few more sentences to people who have not read the previous article.

We added more sentences to clarify the method: *A single one-dimensional (1D) NMR spectrum can resolve few dozens of individual metabolite signals. Due to differences in the NMR properties of small and large molecules, metabolite signals broaden (exhibit reduced intensity) upon protein binding. We exploit this change in signal intensity to detect metabolite-protein interactions.*

Sentence: 1D1H T1rho
Comment: Is this defined anywhere?

We specified the definition of 1D1H in the main text (*'one-dimensional hydrogen-detected'*). The physical explanation of the term "T1rho relaxation" is detailed in the reference citation provided at the end of the corresponding sentence.

Sentence: Water-LOGSY,
Comment: Is this defined anywhere?

We added an explanation for the abbreviation: *water-ligand observed via gradient spectroscopy (Water-LOGSY)*. More detailed explanation is included in our earlier, pilot study, which is cited in the sentence where the term is used.

Comments regarding the results section: Systematic map of protein-metabolite interactions in E. coli central metabolism

Sentence: we calculated the false-positive and …
Comment: Please clarify - How can one tell if something is a false-positive if not all interactions are known yet? That is, how do you know something is false.

For this calculation, we assume that all previously reported interactions (from EcoCyc) are true positives, whereas all other interactions are true negatives. With this assumption, we likely underestimate the total number of true positive interactions, suggesting that the actual True Positive Rate at any given False Positive Rate cutoff is better than suggested by our estimates in the ROC curve analysis (ROC

curve, Appendix Fig S5). We added a reference to the corresponding methods section, where this is explained in more detail.

Sentence: we detected 98 distinct protein-metabolite interactions …
Sentence: We recovered 22 of the 72 previously reported interactions …
Comment: maybe start with this after stating the 98.

We moved this sentence further up, to appear after the statement on '98 interactions …'

Sentence: Piazza 2018, 15.6% recovery
Comment: Better to use 2 significant digits not 3.

Implemented.

Comments regarding the results section: Chemical similarities distinguish between potential allosteric and competitive interactions

Sentence: using Simcomp2
Comment: Maybe write a sentence on what this is and how it works for readers who do not know about it.

We added a sentence to clarify the method: *Simcomp2 identifies the maximal common substructure of two chemical structures using a graph-based method*.

Sentence: Nevertheless, 40% of the NMR-detected interactors have a low chemical similarity (< 0.5) to substrates/products of …
Comment: It would be interesting to see a histogram of the distribution, maybe in an SI figure

We added a reference to Figure 4B after this sentence, to point readers towards the histogram.

Reviewer #3:

The authors studied metabolite protein interactions in the e.coli central metabolism. Therefore 29 proteins were expressed and purified and tested with 4 mixtures containing in total 55 metabolites. The protein-metabolite interactions were analyzed using NMR based methods.
The authors report that they found 98 interactions with 76 being novel. However, only 30% of the known interactions could be verified and only 5 out of 8 novel interactions that the authors scored as allostearic were shown regulatory.

Furthermore the functionality of these interactions was found at extreme high concentrations of the interacting metabolites (e.g. 10mM).

Do the authors see the opportunity that such high concentrations can be achieved in vivo?

To clarify how *in vivo* concentrations relate to the concentrations used in our assay, we added an additional appendix figure (S10) highlighting the concentrations. Additionally, we inserted a statement in the third paragraph of the 'Discussion' section: *"In summary, of the five interactions showing significant effects on enzyme activity, two metabolites show the effect within their physiological concentration range (Zwf-GTP, FbaA-ATP), and three more show the effect at concentrations 1- to 7-fold higher than the anticipated physiological steady state concentration range of the metabolite (Zwf-ATP, FbaA-3PG, FbaA-PEP) (Park et al, 2016; Kochanowski et al, 2017) (Appendix Fig. S10).*"

We would respectfully want to point out though that physiological concentrations might be a somewhat misleading concept for at least three different reasons. Firstly, the reported values are only from a handful of conditions. Secondly, they are steady state concentrations when the cells are in homeostasis. Regulation, however, is expected to occur when cells are pushed out of homeostasis to help them find a new steady state, which is particularly true for the short-term metabolite-protein interactions tested here. It is well-known that dynamically changing concentrations may deviate far out of the steady state concentration ranges. Thirdly, the current estimates of cellular metabolite concentrations assume a uniform distribution of metabolites in cellular cytoplasm, which does not always hold true, as shown by some recent studies (Lohse 2017 Experimental and mathematical analysis of cAMP nanodomains). Since these consideration venture far beyond what we report, we prefer to not discuss them in the manuscript. Nevertheless, we fully agree that in vivo relevance is the now arising question from this work – actually also for the many already reported interactions – which goes far beyond *in vitro* enzyme assays. Our future work we will therefore follow-up in the suggested direction to assess *in vivo* functionality of some of the here detected interactions.

Why could the known interactions not be retrieved?

We would like to point out that by increasing the false-positive rate cutoff, retrieval of over 60% of known interactions could be achieved (see ROC curve in Appendix Figure S5). However, in order to minimize false-positive interactions, we opted for a lower false-positive, and consequently a lower true-positive rate. Additionally, we achieved a 2-fold higher true positive rate than a recent MS-based study (Piazza 2018, 16% recovery of known interactions). So it is not a question of being able to do it or not, but rather one of choice, where we went for smaller numbers at higher-confidence.

In this stage the study is very preliminary and does not lead to novel insights or regulatory principles, yet. The regulatory interactions seem at extreme concentrations, thus the relevance upon 'normal' metabolic regulation may be questioned.

If one would calculate the success of the method to measure the known interactions and the frequency that identified allostearic binders influence the activity of the target enzyme at extreme conditions the the publication is not mature at this stage.

Here, we respectfully disagree with the reviewer's opinion. Firstly, as described above, the discovered regulatory interactions do **not** occur at "extreme" concentrations but rather mostly within a range that could be expected to occur at least during dynamic adaptations, which is precisely the type of situation one would expect metabolite-protein regulation to be relevant. Secondly, we did not set out to identify novel regulatory principles, which in our view is in any case a tough call met only by very few papers. Our clearly defined goal was to probe the depth of our present knowledge of regulatory metabolite-protein interactions in the presently best-investigated biological subsystem. With this single piece of work, we essentially doubled the number of presently known interactions that resulted from literally hundreds to thousands of (wo)man years of hard work. While we are certainly aware of many of the limitations of our work – such as, for example, demonstrating the *in vivo* functionality for all these interactions – we strongly feel that we demonstrated the potential of the method and significantly contributed to completing our knowledge on the regulatory interaction topology in central metabolism. We hope that the reviewer can agree that although the manuscript does not answer all questions, it did achieve its stated goal and is a major step towards at least topological understanding.

Accepted 31st July 2019

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

THE EMBO JOURNAL

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

Corresponding Author Name: Yaroslav Nikolaev, Uwe Sauer
Manusript Number: MSB-19-9008

Reporting Checklist For Life Sciences Articles

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript (see link list at top right).

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars only for independent experiments and sample sizes where the application of statistical tests is warranted (error bars should not be shown for technical replicates)
- when n is small (n < 5), the individual data points from each experiment should be plotted alongside an error bar.
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation (see link list at top right).

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

Please fill out these boxes

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For NMR data the combination of two T1rho measurement replicates, with false-positive rate analysis at 5% false-positive rate cutoff, was considered sufficient to select high-confidence interaction hits. For in vitro enzyme assays the standard triplicate measurements were considered sufficient.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	In Figure 5 we report the mean and s.e.m., the exact statistical test performed is indicated.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The Shapiro-Wilk test was used to confirm normality of the distributions for in vitro enzyme assay data. (see section 'Statistics').
Is there an estimate of variation within each group of data?	Variation was estimated and compared using Bartlett's test (see section 'Statistics').
Is the variance similar between the groups that are being statistically compared?	Yes, Bartlett's test was used to confirm equality of variance between groups. (see section 'Statistics' in 'Methods and Protocols')

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines' (see link list at top right). See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines' (see link list at top right).	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines' (see link list at top right).	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition' (see link list at top right). Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Raw and processed NMR data was deposited in Zenodo (DOI: 10.5281/zenodo.3339911).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The raw data for Figure 4 can be found in Dataset EV2. The raw data for Figure 5 can be found in Dataset EV3. Raw and processed NMR data was deposited in Zenodo (DOI: 10.5281/zenodo.3339911).
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section: Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	See 'Data availability' section in manuscript.
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	See 'Data availability' section in manuscript: The Python code used for NMR experiment setup, spectra processing, calibration and calculation of the difference spectra is available at Github: github.com/systemsnmr/metabolite-interactions. The Matlab code used for identification, signal-to-noise ratio (S/N) quantification, assignment, and disambiguation of interaction hits is available at Github: github.com/systemsnmr/metabolite-interactions.

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA