American Society for
Biochemistry and Molecular Biology
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RE: National Cancer Institute's Request for Information on Soliciting Input on the Use and Reuse of Cancer Metabolomics Data

The American Society for Biochemistry and Molecular Biology is an international nonprofit scientific and educational organization that represents more than 10,000 students, researchers, educators and industry professionals. The ASBMB strongly advocates for strengthening the science, technology, engineering and mathematics workforce, supporting sustainable funding for the American research enterprise, and ensuring diversity, equity and inclusion in STEM.

The National Cancer Institute (NCI) published a request for information titled, "Soliciting Input on the Use and Reuse of Cancer Metabolomics Data on Oct 19. The NCI aims to understand how to support privacy, reproducibility and harmonization in alignment with the new National Institutes of Health Data Management and Sharing Policy to improve equitable access to metabolomics data.

We greatly appreciate NCI's engagement of the scientific community to inform its next steps with cancer metabolomics data-management and -sharing as the research community adapts to comply with the NIH data-management and -sharing policy.

The ASBMB public affairs department has been active in recommending equitable data practices to the NIH and the Department of Education that were compiled and expanded upon in a recent letter to the White House Office of Science and Technology Policy.

Given the unique nature of metabolomics data sets, the ASBMB consulted metabolomics and -omics scientists from academia and industry on their specific data management experiences and needs. The ASBMB has identified several areas of concern that should be considered in the future development of tools, guidance and policies regarding metabolomics data sets.

Generating, using and reusing metabolomics data sets

Recommendation 1: NIH must issue more guidance on what level of metabolomic data must be deposited and curated to satisfy the new data-management and data-sharing policy
The ASBMB shares the concern of many scientists that the new NIH data-management and -sharing policy has the potential to place significant burden on individual scientists, laboratories and core facilites that collect large, complex datasets. Because metabolomics is one such field producing highly diverse, complex datasets, the ASBMB recommends that the NIH issue more guidance on what level of data and information is required to be compliant. Importantly, these new clarifications also must remain sufficiently flexible to accommodate the diverse methods of collection and their individual technical limitations and/or assumptions.

Due to the high complexity and expert knowledge required to analyze and assess metabolomics data, the majority of the experimental data available in metabolomic repositories is not useful to the public or most scientists outside

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the field; nor is it particularly easy to reuse by those within the field. This potentially high burden cost to investigators without much utility poses significant barriers for these scientists to comply with the new NIH data-management and -sharing policy.

Recommendation 2: NIH and NCI should continue to improve the deposition and retrieval process of repositories
The currently available software and tools for deposition and retrieval of metabolic data are cumbersome. In fact, metabolomics researchers are reluctant to extensively deposit their data into a repository due to the onerous effort required and lack of clear utility for the deposited data. To improve the deposition and retrieval process, we recommend that NIH and NCI ensure that repositories, such as the NIH Common Fund's National Metabolomics Data Repository, have the following attributes:

(1) streamlined to minimize the burden of deposition and protect scientists' valuable time and effort
(2) updated to be compatible with stabile isotope tracer datasets
(3) regulated to require only the data and metadata necessary to comply with the policy in a format that supports sustainability in a rapidly evolving field (*i.e.,* data on some file types from more than a decade ago are already inaccessible)
(4) structured to be sufficiently flexible so as to accommodate new technologies in the field and incorporate new functionalities with ease
(5) Embedded with thorough instructions on how to properly retrieve data to ensure they are correctly processed and analyzable by nonexperts

Recommendation 3: NCI should factor in the experimental challenges of metabolomics data collection as they move toward the goal of reuse
Experimental variations contribute to a lot of uncertainty in reusing metabolic data. The individual instrumentation, chromatography columns and sample preparation methods that are utilized will produce unique spectra and must be standardized within an experiment. It is extraordinarily difficult to standardize across the whole metabolomics field. For example, standardization committees coordinated through NIH (*e.g.,* mQACC) currently are addressing standards for LC-MS only. Additionally, the metabolic content from cell extracts can (1) vary based on extraction method (which varies significantly across the field and biases the number of recoverable metabolites) and (2) rapidly change during sample preparation, potentially skewing the data.

Recommendation 4: General pathway software tools need improvement
Another area of concern for metabolomics researchers is the use of metabolic pathway analysis software. These tools can be an excellent starting point but are highly reductive and can lead to significant misinterpretations. Because metabolism varies by tissue type and organism, the results of general pathway software can be highly inaccurate. It must be clearly communicated to users that these tools generate hypothetical outputs that must be validated and not taken as evidence.

Recommendation 5: NIH must establish clear nomenclature for metabolites
The lack of clarity regarding chemical and metabolite nomenclature is another barrier to the use and reuse of metabolomics data. There still remains some debate in the field as to what is considered a metabolite. For example, is a protein or nucleic acid a metabolite? Futhermore, there are considerations around exogenous versus endogenous and interorganismal transformations. The NIH should provide a clear definition for what it considers a metabolite.

Additionally, there are several different standardized formats used to identify and distinguish one chemical from another, *e.g.,* InChIKey, SMILES, PubChem, ChemSpider, CHEBI and several others. The lack of consistency in chemical names can create confusion and difficulty in communicating and reusing data. The ASBMB encourages more standardization of chemical naming in a manner that works for metabolomics as well as across scientific fields. In metabolomics, InChIKey and SMILES were reported as the current front-runners, but they are not fully

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compatible with tracer studies. As progress is made on this barrier, the NCI and NIH should prioritize interoperability between format types.

The data and metadata most necessary to reproduce results reported by metabolomics studies

Recommendation 6: Require metadata for metabolomics data deposition
Metadata are the information necessary to understand the context of experimental data, such as experimental design, sample preparation and equipment details. Without this information, metabolomics data loses significant value and reduces confidence and reproducibility. Alarmingly, certain metabolomics repositories do not require deposition of this information. In contrast, amending raw data with metadata has the potential to be an inordinately burdensome process. The ASBMB recommends requiring *a reasonable degree* of metadata that is standardized in format and interoperable with international standards.

Due to the complexity of some datasets, especially multiomics ones, the ASBMB recognizes that reporting the metadata in a consistent and retrievable manner will be quite challenging and continued engagement from stakeholders will be critical.

Recommendation 7: The NIH and NCI should carefully balance the necessity of data and metadata for validity with the utility and the burden cost to researchers.
The minimum metadata required to reproduce a metabolomics experiment would include information on the instrument platform and settings, chromatography columns used and mobile phase program (if used), and experimental design (including sample treatment, drugs/inhibitors, tracer description, solute preparation, media information and internal standards, when applicable).

The data that are most necessary for reproducing a metabolomic study would of course vary depending on what type of data, *e.g.*, mass spectrometry data or nuclear magnetic resonance (NMR) spectroscopy data. In the case of mass spectrometry, the essential data would include both MS1 and MS2 spectra, indication of data-dependent or data-independent collection, the area under the curve, its transpose and all identifiers and/or annotations. For NMR, the information provided should include the observed spectra and/or the chemical shift data, the peak areas, coupling patterns for all observed nuclei, and concentration of the internal reference standard, which can be used to identify and quantify a metabolite.

The researchers to whom the ASBMB spoke were divided on whether or not to include unknown peaks in metabolomics datasets. The chemocentric approach—providing data on the only metabolites that can be identified at a certain level of confidence—would be the most practical while also making the data easier to reuse and view with respect to the biological significance. Alternatively, the umbrella approach of depositing all spectra, regardless of confidence in identity, has potential utility in the future as technology and computational methods become more sophisticated and new breakthroughs occur. The ASBMB recommends that NIH thoroughly engage the community of stakeholders and experts in metabolomics on this topic to thoughtfully determine the best course of action.

Considerations for selecting and using software and informatics tools for metabolomics

The researchers ASBMB consulted did not overwhelmingly consider any software to be the "standard," in fact, they shared that many labs, core facilities and industrial providers have proprietary software. The frequent use of proprietary software may signal that the currently available software is either (1) too basic to be amenable to unique experimental conditions, (2) too complex and unnecessarily clunky, or (3) too erroneous to produce reliable results.

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Considerations for incorporating metabolomics data into multiomics studies

The validity and feasibility of incorporating metabolomics data in multiomics studies is a rapidly evolving area of research that requires further development. The most significant challenge in incorporating metabolomics is data integration, *i.e.*, normalize the spectral data from mass spectrometry and/or NMR to the output of other -omics techniques, such as genomics, epigenomics, transcriptomics, proteomics and microbiomics. Additionally, -omics data are often heterogeneous and difficult to compare between datasets readily available in repositories. The ASBMB recommends development of gold standards for data collection, reporting, analysis and nomenclature to aid the integration of metabolomics data in multiomics studies. Developing these gold standards will require time and input from stakeholders across multiple fields.

Considerations for how the interoperability of different file formats has promoted or impeded the reuse metabolomics data

ASBMB metabolomics researchers consider the .mzXML file format to be the most commonly used and generally accepted for mass spectrometry data. They noted that this file type can be converted to other file formats and is amenable to future data mining. The use of .csv files to present chemocentric data summaries of identified metabolites is also common.

Final considerations

The current RFI serves as a great first step, but it shouldn't be the last. Developing appropriate data-sharing standards will require substantial time and input from stakeholders across the metabolomics field and should not be tackled solely through the lens of cancer metabolomics. A summit with stakeholders — metabolomics experts in industry and academia and the journals that publish metabolomics research — should be convened for candid and detailed discussions for setting standards and implementing those standards into research workflows. It is critically important for the field of metabolomics that this process be conducted thoughtfully and deliberately. Decisions on these policies must consider both the utility of deposited data and the financial and time costs associated with meeting the requirements.

The ASBMB manages and publishes a gold open-access journal *Molecular & Cellular Proteomics* to foster the development and application of proteomics in both basic and translational research. MCP requires annotated spectra for all proteins that were identified on the basis of one unique peptide or by peptide mass fingerprint, as well as proteins that contain posttranslational modifications. To assist the community in complying with our standards, MCP developed thoughtful, robust guidelines for providing access to annotated spectra.

Experts involved with ASBMB and MCP are available to answer any questions or provide clarifications to any of the above information. To do so, please contact Director of Public Affairs Sarina Neote at publicaffairs@asbmb.org.