An ISA-Tab specification for protein titration data exchange

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Abstract

Data curation presents a challenge to all scientific disciplines to ensure public availability and reproducibility of experimental data. Standards for data preservation and exchange are central to addressing this challenge: the Investigation-Study-Assay Tabular (ISA-Tab) project has developed a widely used template for such standards in biological research. This paper describes the application of ISA-Tab to protein titration data. Despite the importance of titration experiments for understanding protein structure, stability, and function and for testing computational approaches to protein electrostatics, no such mechanism currently exists for sharing and preserving biomolecular titration data. We have adapted the ISA-Tab template to provide a structured means of supporting experimental structural chemistry data with a particular emphasis on the calculation and measurement of pKₐ values. This activity has been performed as part of the broader pKₐ Cooperative effort, leveraging data that has been collected and curated by the Cooperative members. In this article, we present the details of this specification and its application to a broad range of pKₐ and electrostatics data obtained for multiple protein systems. The resulting curated data is publicly available at http://pkacoop.org.

Introduction

The preservation and curation of scientific research data has been a topic of discussion since the 1990s [1,2]. In recent years, increased focus has been placed on the standards and storage needs for scientific research. In most scientific datasets, there are two broad categories of data. Ephemeral data is irreplaceable and cannot be regenerated while stable data can be reproduced from source ephemeral data [3]. The focus of this paper is the metadata associated with the ephemeral data. Metadata is a common type of data that includes the procedures necessary in order to produce the experimental data, qualitative descriptors, persons and institutions involved, and ontological tags providing semantic information for these data. Given ephemeral metadata, stable data can be regenerated via computation, simulation, or reproduction of the analysis as prescribed in the metadata. Ephemeral data, therefore, has intrinsic value for broader meta-analyses of related experimental data sets and reproduction of experimentally observed results [3]. Therefore, there is a significant need to curate data and metadata. The careful documentation and storage of ephemeral data (e.g., software used, laboratory parameters, data, etc.) is an important step toward addressing the reproducibility of stable, computational experimental outcomes.

This paper describes the development of a standard to ensure the preservation and sharing of pKₐ Cooperative data. There currently exist several application-specific data curation efforts that have resulted in standards that specify how data should be preserved; e.g., the Minimum Information About a Microarray Experiment (MIAME) [4] and the Systems Biology Markup Language (SBML) [5]. However, given the diversity of experimental and computational methods used to generate protein pKₐ data, we wanted to use a template that was easily extensible, available as open source, and already used in multiple applications. The schema we identified as the most applicable for this study is the Investigation-Study-Assay Tabular (ISA-Tab) format, which is an open-source standard for biological experimental data [6,7]. ISA-Tab was originally designed for microarray data, but has been expanded to include a wide range of experimental methods relevant to protein biophysics [8], experimental data in biomedicine, nanomaterials [5], and targeted bioinformatics research on stem cells in the Stem Cell Discovery Engine (SCDE) [9]. The SCDE example is relevant to our current work extending ISA-Tab to experimental and computational research. SCDE is an analytical suite with a knowledge base that wraps experimental data using the ISA-Tab specification. This use of the ISA-Tab schema demonstrates its flexibility in configuration, generalized format of data files, as well as adaptability to biological and chemical data curation uses.

We aim to extend the ISA-Tab specification, in a manner similar to the SCDE implementation, for the measurement and calculation of acid dissociation constants (pKₐ-s) in proteins. The acid dissociation constants of protein residues are relevant to biochemistry and biophysics because they reveal important information about protein energetics,
stability, and function. The importance of accurately capturing these values is discussed at length in the pKₐ Cooperative article which also describes the origins of our curated data [10].

Methods

ISA-Tab-formatted (meta)data is organized in a directory containing tab-delimited text files linked by prefixes in the file names [6]. These text files are divided into three categories: Investigation, Study, and Assay with the filename prefixes “i”, “s”, and “a”, respectively. The tab-delimited text files can be read by a lightweight Java interpreter called the ISAcreator or by a wide range of other parsers. The ISAcreator interpreter parses the ISA-Tab-formatted directory of files based on XML configuration files. The configuration file can be modified by direct editing of the XML or via the ISAcreator configurator software. The ISA-Tab file is static, allowing reuse across multiple datasets. The ISAcreator and ISAcreator configurator tools are open source tools, easily accessed through their home page [7].

We expanded the ISA-Tab configuration file by adding additional assay configurations for assays measuring chemical shift, titration curve measurements, pKₐ values, and calculated electrostatic properties such as free energy. For the purposes of the pKₐ Cooperative data exchange, one journal article is considered to be one investigation and an individual ISA-Tab directory of data files is created for each article curated.

Our customized ISA-Tab configuration was tested against a published journal article [11] from the García-Moreno lab that uses both NMR spectroscopy and continuum electrostatics methods to measure/calculate pKₐ values for internal residues of a hyperstable variant of staphylococcal nuclease. An ISAcreator view of the investigation file is shown in Figure 1 displaying information extracted from the García-Moreno journal article. The investigation file contains several important elements: (A) titles of the studies, researcher contact information, a summary of work, and date of publication; (B) pointers to the various study assay files in the Study Assay section; and (C) study design descriptors from several ontologies, including the Ontology for Biomedical Investigations (OBI) [12], the National Center for Biotechnology Information (NCBI) Taxonomy [13], and the National Cancer Institute (NCI) Thesaurus [14].

The left hand column in Figure 1 displays a list of all study and assay files associated with the investigation.

![Figure 1. ISAcreator view of investigation file.](image)

(A) titles of the studies, researcher contact information, a summary of work, and date of publication; (B) pointers to the various study assay files in the Study Assay section; and (C) study design descriptors from several ontologies.
An investigation generally includes several studies; individual study files can be viewed in ISAcreator by selecting items from the lefthand side of the screen shown in Figure 1. An example ISAcreator study file view is shown in Figure 2 and includes (A) a reference molecule ID (if available) for the protein under study and (B) a list of a list of all proteins and variants examined in the study. In the current example, the PDB ID is used as the molecule reference in the Source Name field; however, other IDs (GenBank, etc.) could be used as well with the appropriate references added to the Comment[Source] field. Mutants are described by the Characteristics[organism] field and given unique identifiers in the Sample Name field. The left hand column in Figure 2 displays a list of assay files associated with the study.

Figure 2. ISA-Tab view of study file. (A) a reference molecule ID (if available) for the protein under study and (B) a list of a list of all proteins and variants examined in the study.

A study generally includes several assays; individual assay files can be view in ISAcreator by selecting items from the lefthand side of the screen show in Figure 2. An example ISAcreator assay file view is shown in Figure 3 and includes (A) a single measurement technology or software simulation outcome for (B) a single protein variant identified in the parent study file using (C) a specific measurement protocol to obtain (D) a $pK_a$ value and (E) standard deviation as well as (F) other derived values. A number of ephemeral data attributes are exposed for this assay, including the protein residue (available in the Extract Name and the Protocol REF fields. The Protocol REF field includes an ontology concept to reference the experimental (or computational) approach used for each measurement as well as methods such as linkage analysis used to derive $pK_a$ values from the data. Several protocols have been implemented into our configuration file, including both computational and experimental methods (described in the Results section below). In addition to capturing as much ephemeral non-reproducible data as possible; e.g., additional fitting parameters used in data analysis in Parameter Value[Derived Experimental Data] and associated fields such as linkage (or Hill) coefficients.

Figure 3. ISA-Tab view of assay file. (A) A single measurement technology or software simulation outcome for (B) a single protein variant identified in the parent study file using (C) a specific measurement protocol to obtain (D) a $pK_a$ value and (E) standard deviation as well as (F) other derived values.
Results and discussion

We tested our extended ISA-Tab data-sharing format by curating information from 20 published manuscripts with protein titration data. These manuscripts included NMR spectroscopy \[11, 15 –29\], UV circular dichroism \[30, 31\], and continuum electrostatics calculations \[11, 18, 21, 24, 26, 29, 31, 33\]. To increase the diversity of the test dataset and test generalizability beyond p\(K_a\) values, we also included electrochemistry data of heme proteins \[32\]. The curation of both experimental and computational data is an important aspect of this work. The p\(K_a\) Cooperative recently led a blind prediction challenges for p\(K_a\) data given small amounts of seed experimental information \[34, 35\]. Meaningful analysis and comparison \[10, 36\] between computational predictions requires a standardized data exchange format that captures relevant metadata. The curated ISA-Tab-formatted versions of these articles as well as the ISA-Tab configuration files can be downloaded from (https://pkacoop.org). The Supporting Information for this manuscript contains a ZIP file of the ISA-Tab materials as well as step-by-step instructions for using the files with the ISAcreator software. This curated data is managed via GitHub due to its open availability and participant-sourced revision process.

In future work, we hope to expand the set of curated data and supported assays as well as extend curation to include p\(K_a\)s measured in small molecule compounds such as pharmaceuticals \[37\]. Future work will also include automatic formatting of CSV spreadsheets from existing data tables into an ISA-Tab compliant format to decrease curation time requirements for current and future research. As minimal requirements for p\(K_a\) data sharing, we recommend that the p\(K_a\) parameter values, statistical characteristics of measurements and fits (e.g., standard errors), fitting models, assay types, residues measured or continuum electrostatics model used be included in reported data. We hope that the ISA-Tab p\(K_a\) data sharing format will enjoy adoption within and beyond the p\(K_a\) Cooperative and serve as a starting point for broader adoption of informatics standards by the molecular biophysics community.
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Supporting information

The ISA-Tab-formatted data, described above, are available for download from the pH Co-operative GitHub web page: [http://pkacoop.org](http://pkacoop.org). A ZIP format is also provided as Supporting Information to this manuscript. The ZIP archive includes the ISA-Tab configuration file described in this paper as well as the individual datasets. The ISAcreator application full version is required to view the ISA-Tab files through the customized user interface rather than as spreadsheets; this software is freely available from [http://www.isa-tools.org/software-suite/](http://www.isa-tools.org/software-suite/). After creating an account in ISAcreator, users can add the isaconfigPChem directory to the list of available ISAcreator configurations. Alternatively, users can copy this directory to their ISAcreator installation directory (under Configurations) so that it will be available by default. The provided pH Co-operative ISA-Tab (found in the isa-tab-data directory of Supporting Information) can be loaded through the main menu of the ISAcreator software.