Mass Spec Studio for Integrative Structural Biology

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SUMMARY

The integration of biophysical data from multiple sources is critical for developing accurate structural models of large multiprotein systems and their regulators. Mass spectrometry (MS) can be used to measure the insertion location for a wide range of topographically sensitive chemical probes, and such insertion data provide a rich, but disparate set of modeling restraints. We have developed a software platform that integrates the analysis of label-based MS and tandem MS (MS^2) data with protein modeling activities (Mass Spec Studio). Analysis packages can mine any labeling data from any mass spectrometer in a proteomics-grade manner, and link labeling methods with data-directed protein interaction modeling using HADDOCK. Support is provided for hydrogen/deuterium exchange (HX) and covalent labeling chemistries, including novel acquisition strategies such as targeted HX-MS^2 and data-independent HX-MS^2. The latter permits the modeling of highly complex systems, which we demonstrate by the analysis of microtubule interactions.

INTRODUCTION

Integrative methods in structural biology are delivering impressive visualizations of higher-order multiprotein associations. The functional data gained from such representations are essential for understanding the properties emergent from self-assembling protein "building blocks". The integrative concept involves a high-resolution structural analysis of these building blocks through conventional means, and then leapfrogging their inherent limitations by completing the structure-building exercise using biophysical methods, which may be of lower resolution, but can be applied to the assembled state (Karaca and Bonvin, 2013; Thalassinos et al., 2013; Ward et al., 2013). The potential of this approach has been portrayed through the modeling of a growing number of complex states, built from fitting the refined structures of individual components into cryoelectron microscopy reconstructions (Schraidt and Marlovits, 2011; Topf et al., 2008), as well as small-angle X-ray scattering envelopes (Devarakonda et al., 2011; Putnam et al., 2007). Models can generate testable mechanisms even when the structures of all the building blocks are not fully available, as shown in a recent structure for a membrane-bound proton-driven ATP synthase (Lau and Rubinstein, 2012). Any technology that contributes spatial or conformational information on the free and bound states adds considerable value to accurate model building, and when chosen carefully, technologies with complementary attributes can overcome deficiencies in any one approach (Alber et al., 2007; Lasker et al., 2012).

As we continue to image molecular events at wider spatial and temporal scales, we require methods that can provide restraint data under a wide range of conditions. Biological mass spectrometry (MS) is moving to support such activities and is quite likely the most promising technology for generating residue-level topographical data in the least restrictive manner (Politis et al., 2014). Numerous recent examples have begun to incorporate MS for structure-building activities. Crosslink detection by proteomic methods and the computational tools developed for them are useful for coarse positioning (Ciferri et al., 2008; Greber et al., 2014; Kahraman et al., 2013; Merkley et al., 2014; Walzthoeni et al., 2013), but a wealth of "single-point" chemistries are available to monitor conformational dynamics and map proteins more completely and at higher resolution (Konermann et al., 2011; Mendoza and Vachet, 2009). MS methods developed to monitor site-specific labeling kinetics can define interfaces at a resolution approaching individual residues (Bennett et al., 2010; Landgraf et al., 2012; Melero et al., 2012; Pan et al., 2012; Roberts et al., 2012). Labeling chemistries are available for both the protein backbone (hydrogen/deuterium exchange) and amino acid side chains (covalent methods like hydroxyl radical labeling).

Label detection by MS shares certain features with MS-driven proteomics. Both invoke enzymatically driven workflows to generate large sets of peptides. These peptides need to be identified and then quantified using either label-based or label-free methods. However, the experiments are structured quite differently and the data are used in much different ways. MS-based integrative methods begin with a known set of proteins, often use different proteases (Ahn et al., 2013), and need to quantify chemical modifications at every residue in a sequence. The data are then interpreted for structural or conformational

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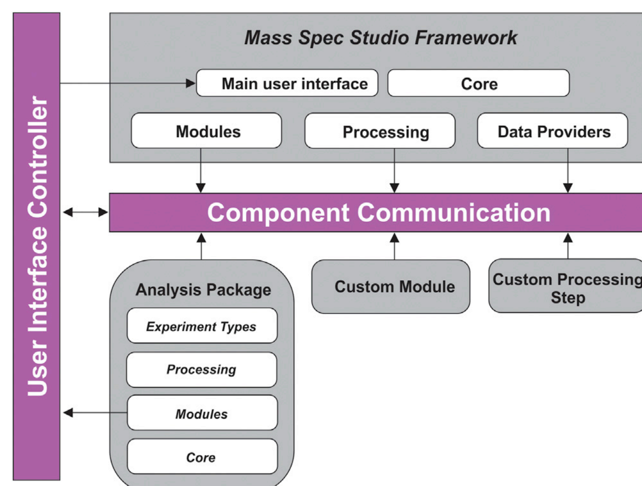

Figure 1. A Conceptual Overview of the Component-Based Architecture of the Studio Framework
The encapsulation model for the core library allows for easy adding, swapping, and removing of components. The component communication model combined with the user interface controller allows seamless linkage of components with each other, as well as with the core library.

meaning. A number of software tools support the basics of hydrogen/deuterium exchange (HX) analysis, for example HDX Workbench (Pascal et al., 2012), Hydra (Slysz et al., 2009), and Hexicon (Lindner et al., 2014), but no platform is sufficiently generic to accommodate any labeling chemistry, or support the ultimate goal of the integrative approach, namely, the restraint-based modeling of molecular structures. Here we present the Mass Spec Studio (the Studio), an adaptable framework designed to support the varied demands of MS-based integrative structural biology. The Studio incorporates efficient processing of liquid chromatography-tandem mass spectrometry (LC-MS/MS) data with workflows designed to support the unique challenges of integrative methods, which includes the extraction of modeling restraints and structure-building activities.

RESULTS

A Framework for Rapid Application Development

Most software for the processing of MS data is vendor-supplied and inflexibly tied to a set of industry-driven applications. Support for structural biology is lacking, both in terms of quantitating chemical labeling events in proteins, and mining the data for structural restraints. The Studio represents a new extensible architecture for the analysis of MS data (Figure 1) and is specifically designed to foster the development of innovative structure-based methods involving MS data. It supports a plugin model, designed to capture and reuse components for a variety of applications. Analysis packages are assembled from a base of components, and novel components are added to a repository for reuse. The Studio presents a flexible framework that automatically links components in the correct fashion, and communication is through high-level interfaces rather than at a low level through source code. An efficient communications protocol allows us to build and concatenate application packages to support entire workflows, spanning data processing to structure building. Essential elements of the design and workflow are provided in Supplemental Experimental Procedures (available online). The Studio offers a series of prebuilt application packages for structural biologists as described below and functions with data from all major instrument platforms. Additional details on application functionality are supplied in Supplemental Experimental Procedures.

HX Analysis Package

There are three types of experiments that are supported by the HX analysis package, all based on peptide-level deuteration analysis, or the bottom-up approach (Marcsisin and Engen, 2010). The method involves continuous labeling of proteins and protein complexes using D_2O. Labeled samples are quenched to arrest the exchange, and then digested with a nonselective protease. The rate of deuterium labeling is measured at various time-points, and a common goal for each experiment type is to determine where and how a complexation event alters the rate of deuterium incorporation at locations in the protein backbone. The experiments differ in how they support complex sample types and in how they use MS/MS data for label measurement.

One-Dimensional HX-MS

The one-dimensional (1D) HX-MS experiment supports the extraction of peptide-level deuteration data from large sets of LC-MS runs. It represents a 1D analysis, in that only MS spectra are used to quantify deuterium incorporation. The workflow is used to visualize binding-induced changes in deuteration kinetics for individual proteins or those involved in larger multiprotein assemblies (Figure 2A). Projects are assembled from all LC-MS data files, together with lists of peptides and their retention times identified in previous experiments using proteomics methods. The deuteration data are then extracted from all the peptides detected in each LC-MS run. The HX analysis package implements an interactive graph control module, to rapidly validate and correct peptide selections and isotope profile definitions (Figure 2B). Correcting peptide lists generated through proteomics experiments is necessary, as such experiments do not mesh cleanly with the demands of HX-MS analysis (Wales et al., 2013). Noisy spectra, strongly overlapped isotopic distributions, and non-apex chromatographic retention times can generate successful peptide identifications, but may not be useful for deuteration analysis. The set of LC-MS runs can be quickly reprocessed using the validated peptide list, to generate a validated data set of deuteration values.

To illustrate the functionality of the Studio for this experiment type, we explored the effect of nucleotide exchange on the conformational status of mitotic centromere associated kinesin (MCAK; see Supplemental Experimental Procedures). MCAK depolymerizes microtubules in a process that is essential for the detection and capture of sister chromatids in the developing mitotic spindle (Wordeman and Mitchison, 1995; Wordeman et al., 2007). This process is driven by conformational changes in the kinesin upon the exchange of ADP for ATP. MCAK conformational stability is strongly regulated by the exchange, promoting a transition between an open (ADP) and a closed (ATP) state (Ems-McClung et al., 2013). Replicate deuteration data for the ADP and ATP loaded forms of a truncated EGFP-MCAK were

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