PNNL Protein Complex Characterization Efforts


Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352
Illinois R. Pitzer, Department of Biology, Washington University, St. Louis, MO 63130

Identifying protein complexes using AMT tags

The database of Shewanella accurate mass and time (AMT) tags provides the basis for high-throughput characterization of protein complexes at either the peptide level or intact protein level. The intact protein level analysis is enabled by the peptide level approach (by providing their intact identifications) and also complements the information obtained at the peptide-level with additional information on protein modifications (e.g., chemical modifications, protein truncation). Regardless, the use of AMT tags can greatly speed the analysis and potentially allow characterization of hundreds of proteins per complex. Peptide coverage by AMT tags is indicated by the pie chart (right).

Value-added protein complex characterization at the intact protein level – initial demonstration

To evaluate our approach, we have initially studied the well-characterized yeast large ribosomal subunit. The 43 proteins in this complex were previously identified at the peptide level using tandem MS, providing an expected set of tentative molecular weights. The constrained level of complexity associated with most protein complexes (ignoring obviously low-level contaminants) allows the detected masses to be assigned to the various proteins, as well as (in most cases) assignment of their modification states.

Advantages of intact protein proteomics:
- Augments peptide level analyses
- Less complex
- Much more information on protein modification states
- Potentially more quantitative, faster, and more sensitive

Higher characterization throughput at lower cost

A component of the PNNL program is to develop an approach that provides both increased confidence, higher throughput, and a quantifiable tool for characterizing protein complexes. We have initially explored the utility of characterizing protein complexes at the peptide level using AMT tags with Q-TOF instrumentation as an alternative to much less sensitive and lower throughput approaches based upon tandem MS (e.g., using ion trap mass spectrometers) or more expensive FT-ICR instrumentation that is needed for much more demanding “whole proteome” analyses.

These results show:
- Q-TOF instrumentation augmented by the use of LC elution time information provides sufficient specificity for application of AMT tag approaches.
- The AMT tag approach with LC-Q-TOF analysis provides sufficient specificity for protein complex characterization, along with high throughput, and preliminary quantitation.
- The use of quantitative information and multiple analyses (e.g., using different wash conditions) will be needed with this approach to better qualify which proteins are part of the complex in contrast to being nonspecifically associated.

Tagged proteins generated to date for pull-down studies at PNNL

Complex generation and isolation

Using recombinant proteins

Biochemical enrichment

Automation (capture column)

Initial results

Initial attempt of pull-down with ptpA

Initial attempt of pull-down with sopA