Experimental design

The experiment used cysteine adducts from iodoacetamide (carbamidomethylation) or acrylamide (propionylation) as known true positives to estimate relative sensitivity and specificity of modified peptides identification using agnostic discovery tools: X!Tandem plus SAMPEI, MSFragger, and Byonic.

Raw MS data are publicly available via the PRIDE repository with dataset identifiers PXD019793.

File naming

Files are named using the following syntax:

> s{*cysteine alkylating agent*}_m{*modification set in conventional searches*}_{*algorithm*}.xlxs

> Cysteine alkylating agent can be *IAA* for iodoacetamide, *ACL* for acrylamide, or *ACLIAA* for a 1:1 mixture.

> Modification can be *IAA* for carbamidomethylation, or *ACL* for propionylation.

> Algorithm can be: *X!tandem* (conventional search), *SAMPEI* (using X!tandem to generate target PSM), *Byonic* (conventional search), *ByonicWT* (Byonic with wild-card feature enabled), *MSFraggerC* (conventional search), or *MSFraggerO* (open search).

> For hybrid searches, the syntax is modified as s{*cysteine alkylating agent*}_{*algorithm used for conventional search*}_m{*modification set in conventional searches*}_SAMPEI.xlxs

Sensitivity of tools for agnostic PTM discovery (Figures 2c and S3)

The spectral pool consisted in a 1:1 mixture of carbamidomethylated or propionylated peptides. Raw spectra were analyzed initially setting either carbamidomethylation or propionylation as variable modifications in conventional closed searches. Agnostic discovery was performed setting one modification (i.e carbamidomethylation or propionylation) as variable, and using MSFragger (open search), Byonic (with wild card function enabled), and SAMPEI (using X!Tandem results as targets) to identify the alternative cysteine adduct. Relative sensitivity was calculated as the fraction (%) of correct agnostically discovered modified peptide-spectral matches compared to modified peptides identified with conventional search.

Hybrid searches (Figure S3)

The spectral pool consisted in a 1:1 mixture of carbamidomethylated or propionylated peptides. Raw spectra were initially analyzed by conventional database-search using ByOnic, MSFragger and MaxQuant setting either carbamidomethylation or propionylation as variable modifications in conventional closed searches. Agnostic discovery was performed using SAMPEI and using as input the PSM sets generated by ByOnic, MSFragger and MaxQuant.

Specificity of tools for agnostic PTM discovery (Figure 2d)

The spectral pool was generated from peptides exclusively carbamidomethylated or propionylated. Raw spectra were analyzed initially setting either carbamidomethylation or propionylation as variable modifications in conventional closed searches. Agnostic discovery was performed under the same conditions and using MSFragger (open search), Byonic (with wild card function enabled), and SAMPEI (using X!tandem results as targets) to identify the alternative (and *bona* fide) absent cysteine adduct.

Relative specificity was calculated as the fraction (%) of incorrect agnostically discovered modified peptide-spectral matches compared to modified peptides identified with conventional search.