Application of SILAC data in antibiotic discovery

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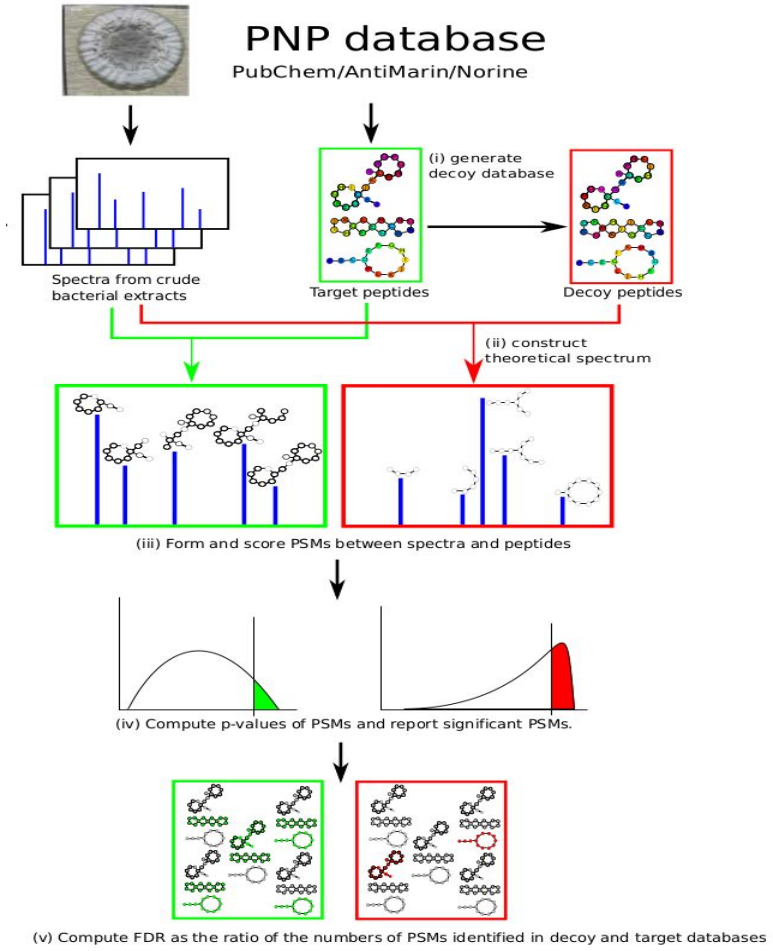
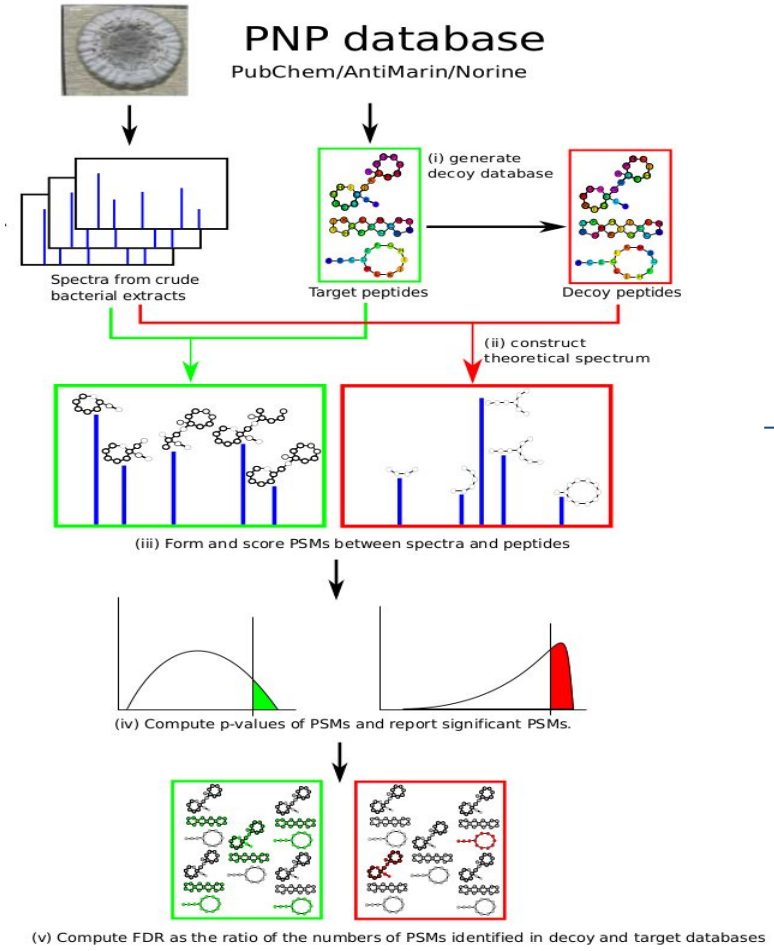
Dereplicator pipeline



Problems



It can identify only a small fraction of PNPs with a high confidence

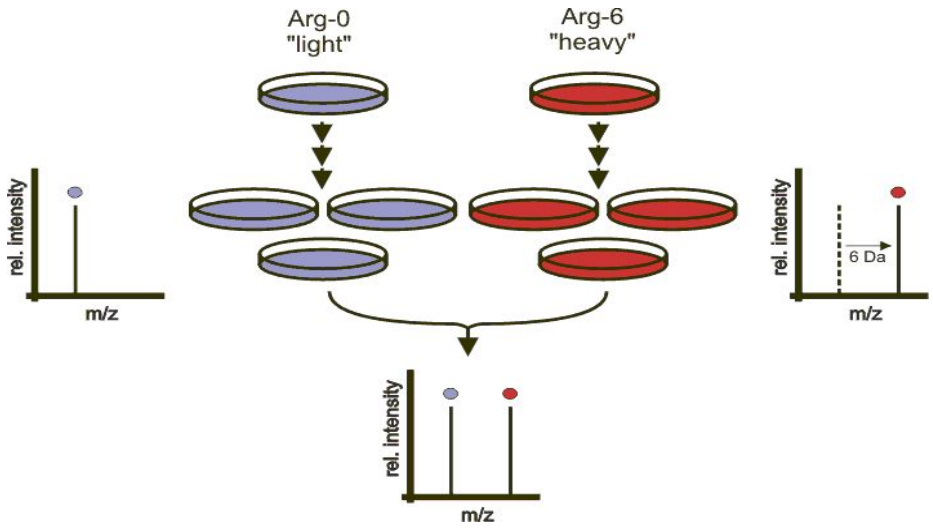
Many correct Peptide-Spectrum Matches (PSMs) are often rejected because their scores and significance (P-values) are mediocre.

Goals

- ✓ Think how to use SILAC data for improving antibiotic discovery
- ✓ Implement scoring procedure for pair of spectra and a given compound
- ✓ Implement the full pipeline
- ✓ Test on real SILAC data and tune the parameters
- ✓ Support RiPPquest output

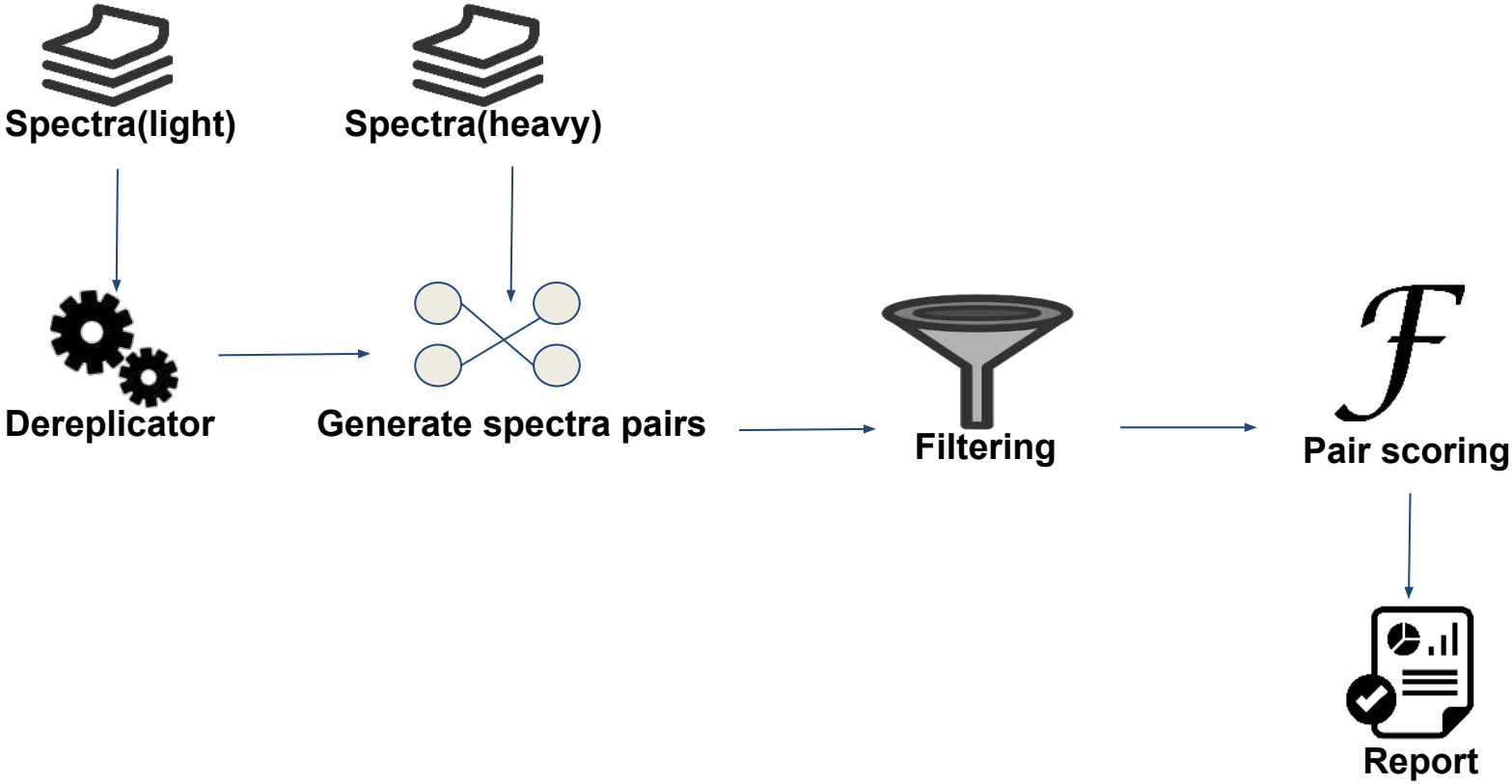
SILAC

Stable isotope labeling with amino acids



- Cells are grown in almost identical culture media
- One contains a 'light'(^{12}C), when the other a 'heavy'(^{13}C) form of a particular amino acid

Pipeline


Spectra(light)
Spectra(heavy)
Dereplicator
Generate spectra pairs
Filtering
$\mathcal{F}$
Pair scoring
Report

Spectra pairs and filtering

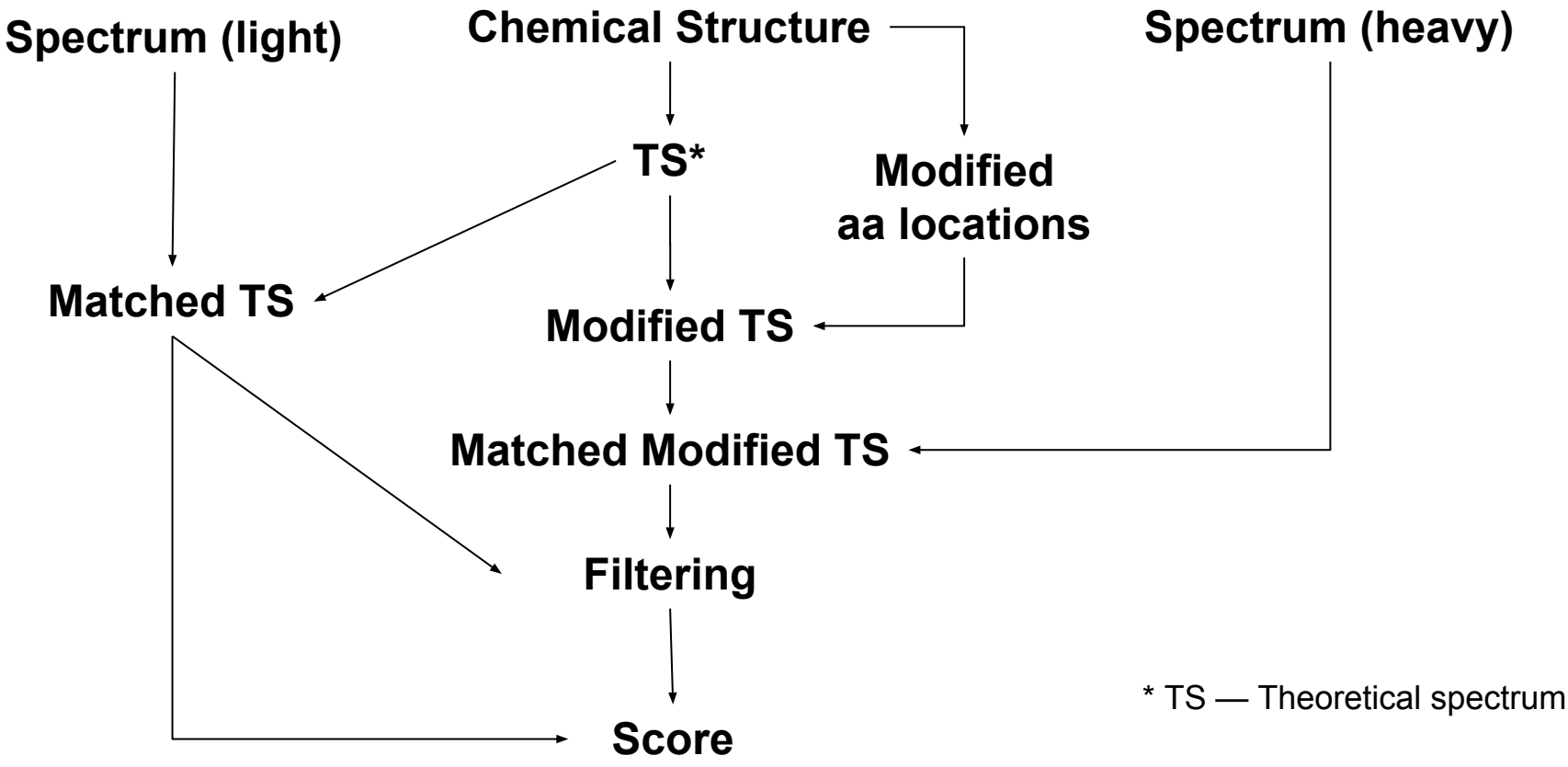
- Get all spectra from:
  - Files with **^{12}C** (reported by Dereplicator)
  - Files with **^{13}C** (all)

- Find such pairs of spectra for which the mass difference is a multiplier of 6 Da (± threshold)

- Drop pairs that do not have enough shared & *shifted** peaks

  * *shifted* means peaks different by k * 6 Da

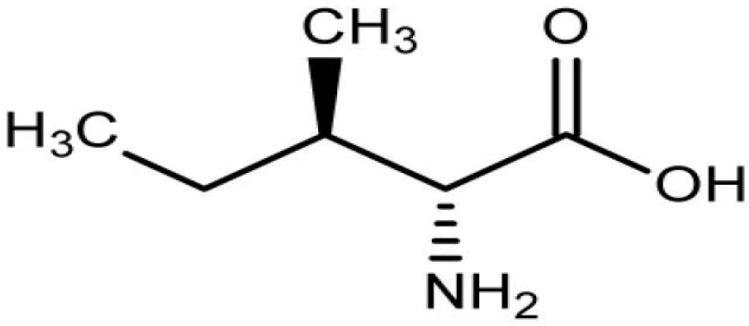
Scoring pipeline



* TS — Theoretical spectrum

Modified aa finding

- Standard amino acids may be represented as binary trees!



Isoleucine

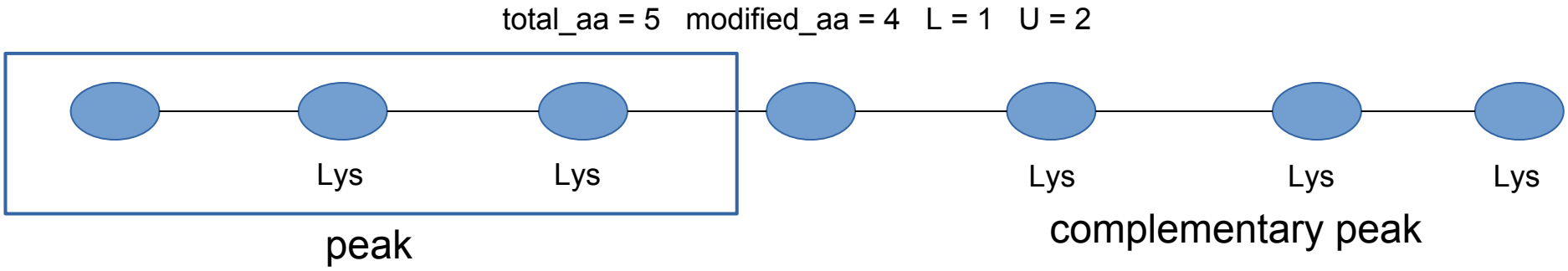
For the aa and each candidate in PNP

1) Remove Hs and reroot at N

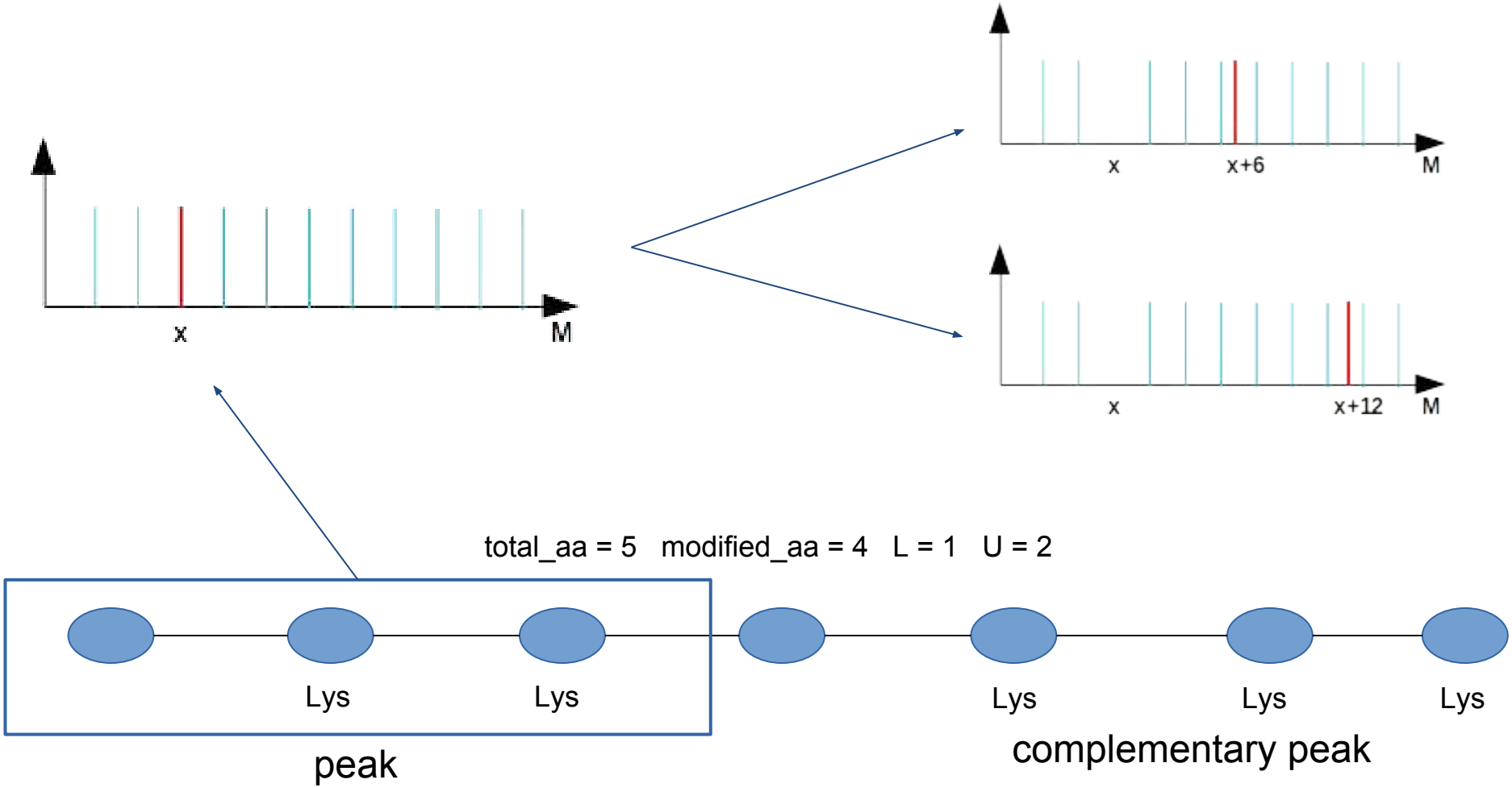
2) Check isomorphism in O(V)

TS modification

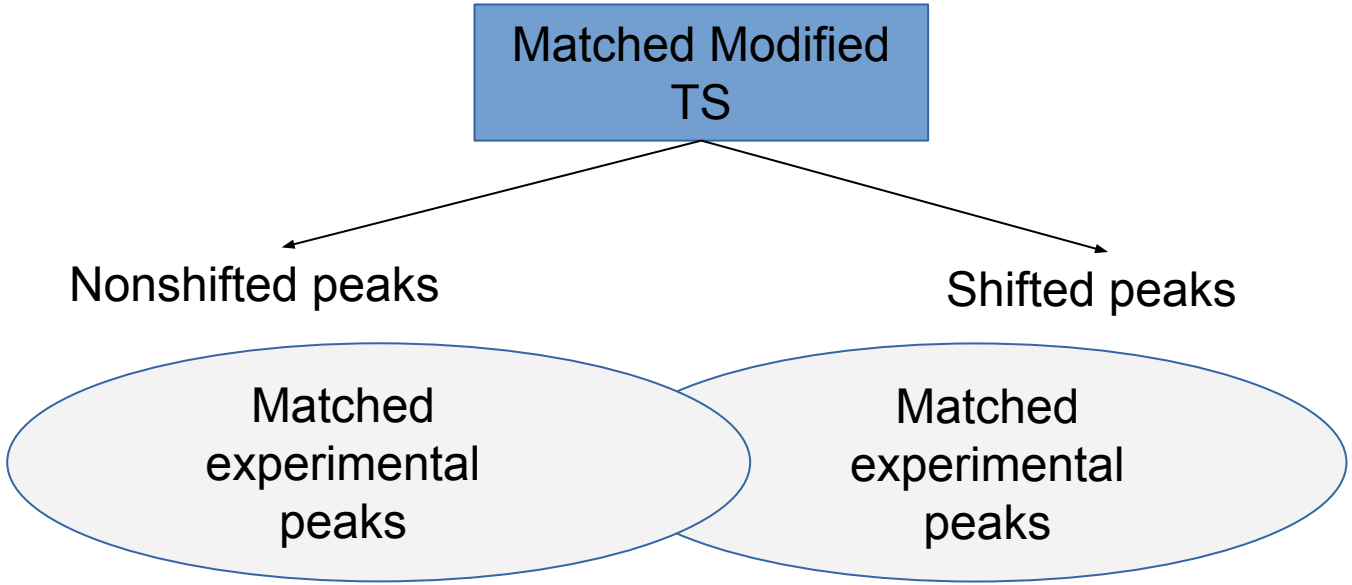
- Input: TS, *Lys* locations, modified_aa, total_aa
- For each **peak** in TS:
  - How many *Lys* included in **peak** (aa_in_peak)
  - L = max[0, modified_aa - aa_in_comp_peak]
  - U = min[aa_in_peak, modified_aa]
  - Add new *shifted* peak for each **i** in [L, U]



TS modification

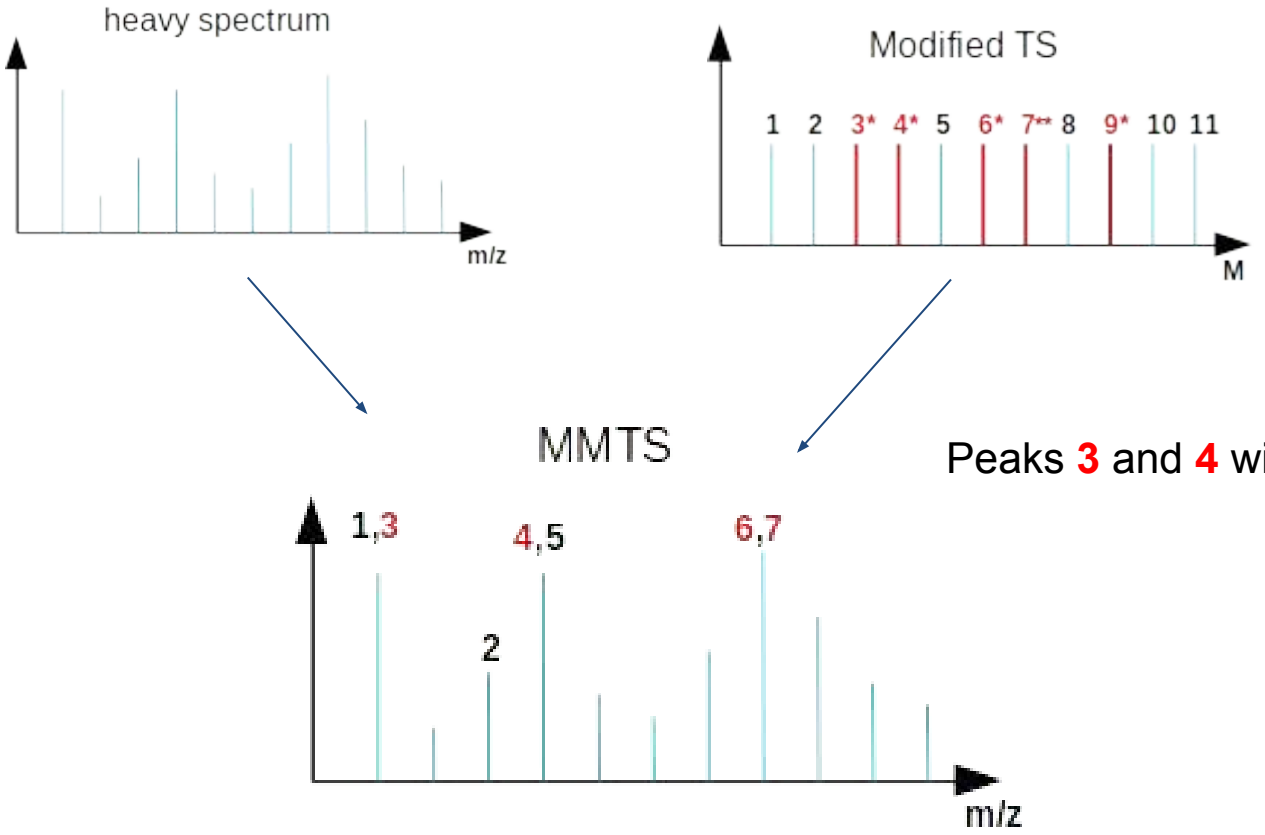


Matched Modified TS filtration



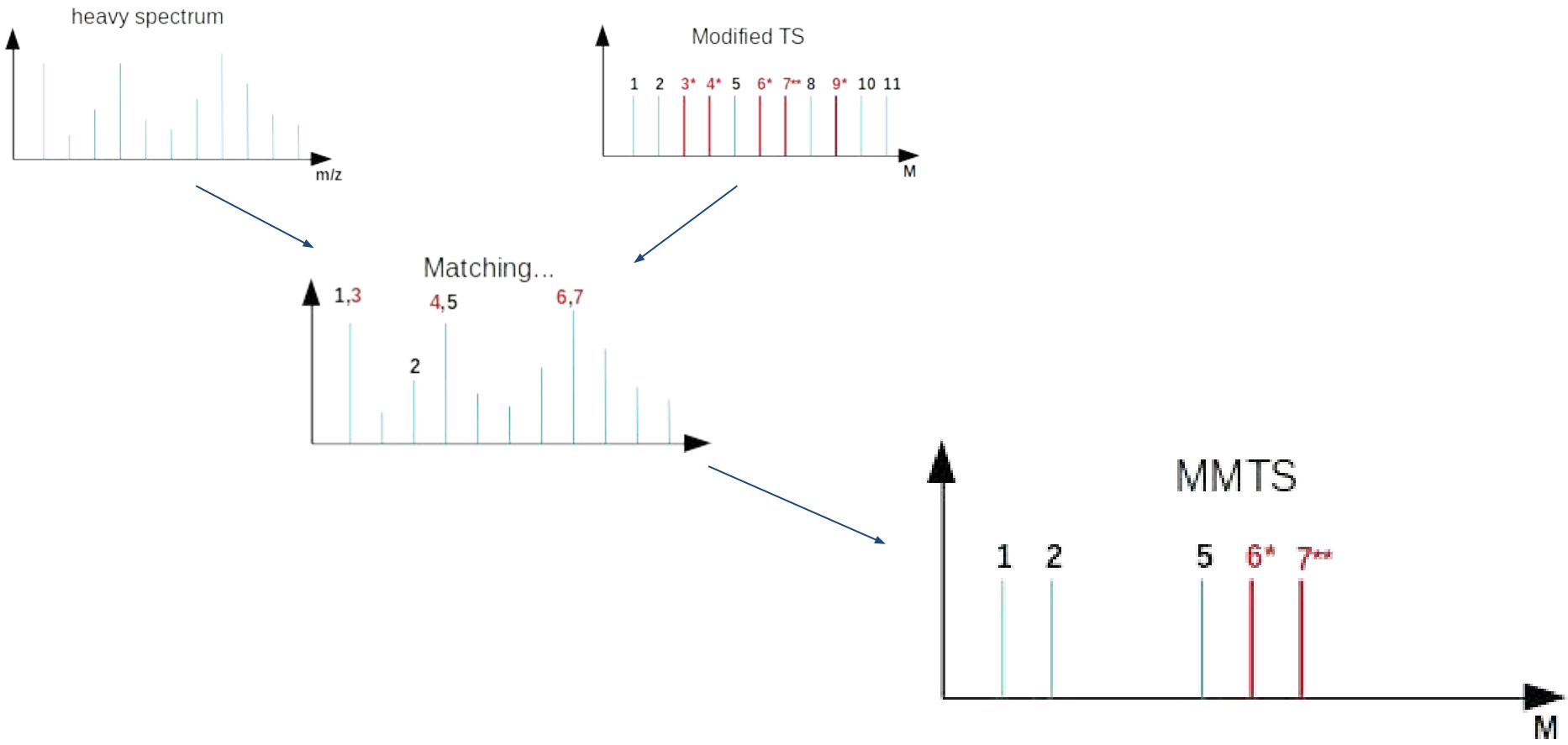
Remove *shifted* peaks matched with experimental peaks explained by *nonshifted* peaks

Matched Modified TS filtration



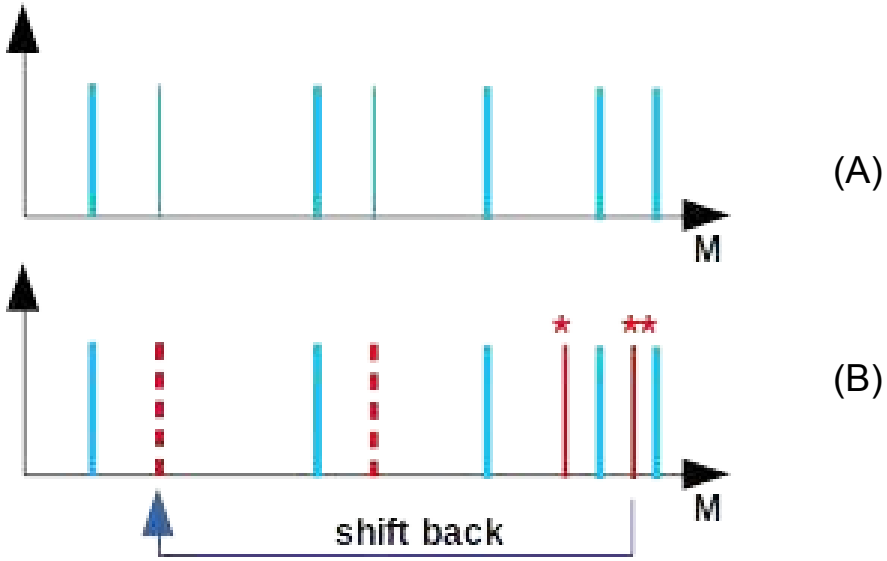
Peaks **3** and **4** will be removed!

Matched Modified TS filtration



Scoring

- Input: Matched TS (A), Matched Modified TS (B)
- Score = (N, S)
  - N is nonshifted score
  - S is shifted score



- N – # nonshifted peaks from B having equivalent peaks in A
- S – # shifted peaks from B having *shifted-back equivalent* peaks in A

Results

- Dataset 1 (Isoleucine-labeled):
  - 77498 heavy spectra and 70451 light spectra
  - 43618 spectra pairs
  - 2477 pairs after filtering
  - 2358 scored pairs
  - 1648 with shifted score >= 10
  - 679 with shifted score in [5, 10)

Results

- Dataset 2 (Lysine-labeled):
  - 14067 heavy spectra and 70451 light spectra
  - 12222 spectra pairs
  - 177 pairs after filtering
  - 108 scored pairs
  - 18 with shifted score >= 10
  - 58 with shifted score in [5, 10)

Thank you.