Probabilistic framework for integration of mass spectrum and retention time information in small molecule identification

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1. Small Molecule Identification in Untargeted Metabolomics

- Challenge in untargeted metabolomics studies: **Identification of the small molecules** present in a biological sample
- **LC-MS^2** widely used analysis platform: Liquid chromatography (LC) coupled with tandem mass spectrometry (MS^2) (Fig. 1)
- Most machine learning approaches for small molecule identification only utilize MS^2 information [4, 3]
- LC retention time (RT) information can aid small molecule identification [6, 8]
- **Challenges utilizing RT information:** (1) LC-system specific RTs and (2) public RT databases are limited in size and molecule coverage

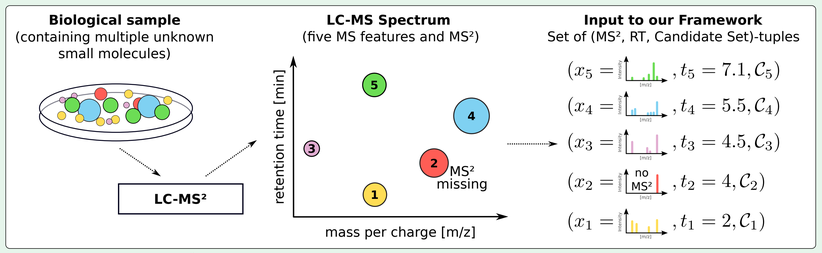


Fig. 1: LC-MS^2 analysis pipeline and resulting data used as input for our framework.

2. Retention Time (RT) Utilization for Small Molecule Identification

1) **Comparison of measured RTs with (in-house) reference RTs**
   - References databases (DB) typically small and LC-system specific
   - RT mapping between LC-systems possible, but requires DB overlaps [8]
2) **Comparison of measured RTs with predicted ones** [1]
   - RT prediction models allow to get RTs for practically every molecular structure
   - Majority of prediction models trained for a single LC-system only
3) **Compare RT-proxy or retention order with observed RT information** [6, 2]
   - Allows for predictions within an whole LC-system family, e.g. reversed-phase
   - Retention orders largely preserved across LC-setups and prediction possible

Our proposed approach:

- Exploitation of all pairwise observed retention orders in an LC-MS^2 datasets
- Comparison of observed and predicted retention orders to down- and up-vote potential molecular structures of the unknown molecules.

3. LC-MS^2 Experiment Data: Input and Output of our Framework

- **Input:** Preprocessed LC-MS^2 data, i.e. after peak-picking and alignment (Fig.1):

$$\mathcal{D} = \{(x_i, t_i, \mathcal{C}_i)\}_{i=1}^{N}$$

  x_i : MS Information; MS^2, or MS^1 (precursor m/z) if no fragmentation available
  t_i : Measured RT
  $\mathcal{C}_i$: Molecular candidate sets, e.g. molecular structures found in PubChem by exact mass search
  N : Number of MS features
- **Precomputed MS scoring assumed:** MS^2 scores, e.g. by CSI:FingerID [4], MetFrag [6] or IOKR [3], or deviation of candidate and precursor mass for MS^1
- **Output:** Ranking of the molecular candidates in $m_{ir} \in \mathcal{C}_i$ for each MS feature i
- Ranking based on MS and RT information

4. Our Probabilistic Framework to integrate MS and RT Information

- **Graphical model** G superimposed on the LC-MS^2 data (Fig. 2)
- Let $G = (V, E)$ be complete graph with a **node** $i \in V$ for each MS feature, and an **edge** $(i, j) \in E$ for each feature pair
- Discrete random variable $z_i \in \mathcal{Z}_i = \{1, \ldots, n_i\}$ associated with each node ($n_i = |\mathcal{C}_i|$)
- Candidate assignment for the complete data $\mathbf{z} = \{z_i \,|\, i \in V\} \in \mathcal{Z}_1 \times \ldots \times \mathcal{Z}_N = \mathcal{Z}$
- Intuitively: Random variable z_i denotes the candidate $m_{ir} \in \mathcal{C}_i$ assigned to feature i.
- Pairwise **Markov Random Field** as probabilistic model [5]:

$$p(\mathbf{z}) = \frac{1}{Z} \prod_{i \in V} \psi_i(z_i) \prod_{(i,j) \in E} \psi_{ij}(z_i, z_j)$$

- Potential functions: $\psi_i(z_i)$ MS score and $\psi_{ij}(z_i, z_i)$ match of observed and **predicted retention order**
- Molecular **candidates ranked** based on max-marginals [5] (Fig. 2):

$$p_{\max}(z_i = r) = \max_{\{\mathbf{z}' \in \mathcal{Z} \,|\, z'_i = r\}} p(\mathbf{z}')$$

- Intuitively: Maximum marginal probability of a candidate assignment $\mathbf{z}$ with $z_i = r$.

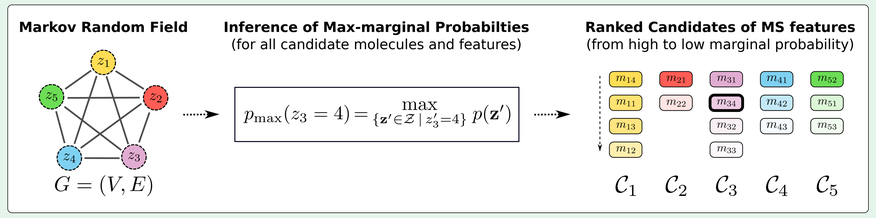


Fig. 2: MRF probability distribution and candidate ranking, e.g. MS feature $i = 3$ and candidate 4 (m_{34}).

5. Encoding MS and Retention Order Information: ψ_i and ψ_{ij}

- Goodness of the candidate assignment $\mathbf{z}$ modeled by potential functions ψ (Fig. 3)
- Node potential $\psi_i : \mathcal{Z}_i \to \mathbb{R}_{>0}$: $\psi_i(z_i = r) = f(x_i, m_{ir})$
  - f returns the MS matching score $\in (0, 1]$ of spectrum x_i and candidate m_{ir}
- Edge potential $\psi_{ij} : \mathcal{Z}_i \times \mathcal{Z}_j \to \mathbb{R}_{>0}$, with σ being the sigmoid function:

$$\psi_{ij}(z_i = r, z_j = s) = \sigma(\underbrace{\text{sign}(t_i - t_j)}_{\text{observed retention order}} \cdot \underbrace{\mathbf{w}^T(\phi(m_{ir}) - \phi(m_{js}))}_{\text{predicted retention order}})$$

- Intuitively: Matching observed and predicted retention orders receive high scores.
- **Retention order prediction** using Ranking Support Vector Machine (RankSVM) $\mathbf{w}$ [2]
- Candidate molecules m_{ir} representation using non-linear features ϕ

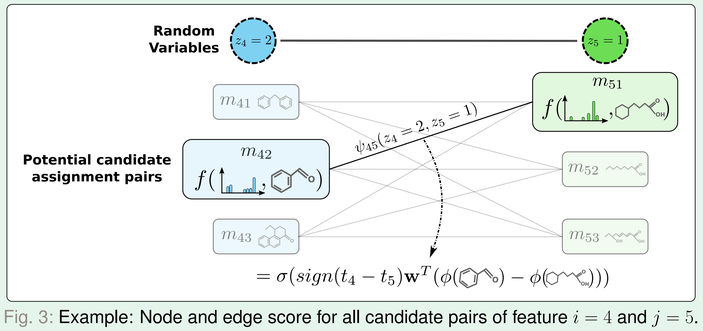


Fig. 3: Example: Node and edge score for all candidate pairs of feature $i = 4$ and $j = 5$.

6. Experiments and Results

- **Evaluation datasets:** CASMI 2016 [7], EA subset from MassBank used by [6]
  - 681 (MS^2, RT)-tuples with median number of candidates between 120 and 919
  - Datasets cover two different LC columns and flow gradients
- **RankSVM training data:** 1248 RTs from PredRed [8] and CASMI 2016 training
  - No evaluation set molecule in RankSVM tranining set
- **Performance measure:** Top-k accuracy, percentage of correct molecular candidates at rank $\leq k$

Experiment 1: Comparison to MetFrag + LogP (RT Proxy) Prediction

- MetFrag relaunched [6]: Prediction of LogP values for candidates, linear model mapping measured RTs to LogPs, candidate re-ranking based on LogP deviation

Method	Top-1	Top-5	Top-10	Top-20
MS^2 + RT (Our)	21.3	52.9	64.0	74.3
MS^2 + RT (MetFrag & LogP)	20.5	49.1	61.2	72.6
Only MS^2 (baseline)	16.7	49.5	60.4	70.6

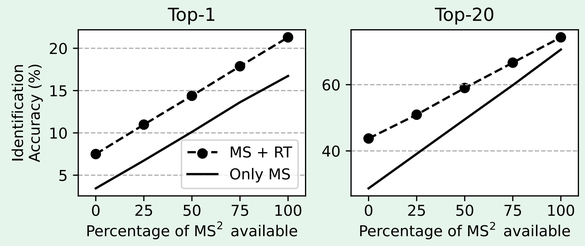
Experiment 2: Performance with different MS^2-Scoring Methods

- MetFrag (in-silico fragmenter scores) and IOKR [3] as MS^2-scoring methods

MS^2-Scorer	Method	Top-1	Top-5	Top-10	Top-20
MetFrag	MS^2 + RT (our)	21.3	52.9	64.0	74.3
	Only MS^2 (baseline)	16.7	49.5	60.4	70.6
IOKR	MS^2 + RT (our)	26.7	52.1	62.5	70.3
	Only MS^2 (baseline)	25.1	49.5	60.3	67.6

Experiment 3: Missing MS^2 Spectra

- Simulating missing MS^2 information: Varying from 0% to 100% MS^2
- If only MS^1: Use mass deviation between precursor and candidate molecule



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