Code available

Check out the paper

Contact us

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

Eric Bach [1,✉], Simon Rogers [2], John Williamson [2], and Juho Rousu [1]

[1]Department of Computer Science, Aalto University, Espoo, Finland, [2]School of Computing Science, University of Glasgow, Glasgow, UK

1. Small Molecule Identification in Untargeted Metabolomics

- Challenge in untargeted metabolomics studies: **Identification of the small molecules** present in a biological sample
- **LC-MS²** widely used analysis platform: Liquid chromatography (LC) coupled with tandem mass spectrometry (MS²) (Fig. 1)
- Most machine learning approaches for small molecule identification only utilize MS² 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

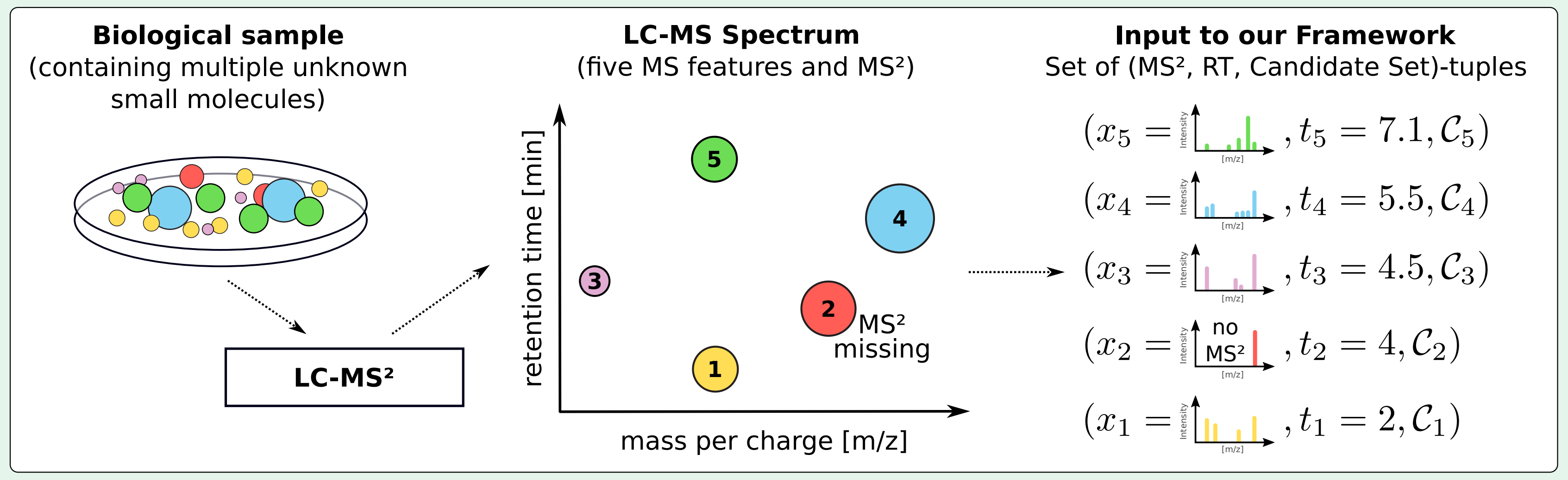

Biological sample
(containing multiple unknown small molecules)
LC-MS²
LC-MS Spectrum
(five MS features and MS²)
retention time [min]
mass per charge [m/z]
5
4
3
2
1
MS² missing
Input to our Framework
Set of (MS², RT, Candidate Set)-tuples
$(x_5 = \ldots, t_5 = 7.1, \mathcal{C}_5)$
$(x_4 = \ldots, t_4 = 5.5, \mathcal{C}_4)$
$(x_3 = \ldots, t_3 = 4.5, \mathcal{C}_3)$
$(x_2 = \ldots, t_2 = 4, \mathcal{C}_2)$
no MS²
$(x_1 = \ldots, t_1 = 2, \mathcal{C}_1)$
Intensity
[m/z]

Fig. 1: LC-MS² 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-MS² datasets
- Comparison of observed and predicted retention orders to down- and up-vote potential molecular structures of the unknown molecules.

3. LC-MS² Experiment Data: Input and Output of our Framework

- **Input:** Preprocessed LC-MS² 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; MS², or MS¹ (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:** MS² scores, e.g. by CSI:FingerID [4], MetFrag [6], or IOKR [3], or deviation of candidate and precursor mass for MS¹
- **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-MS² 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 $\mathbf{z} = \{z_i \mid i \in V\} \in \mathcal{Z}_1 \times \ldots \times \mathcal{Z}_N = \mathcal{Z}$
- 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 $z_i = r$.

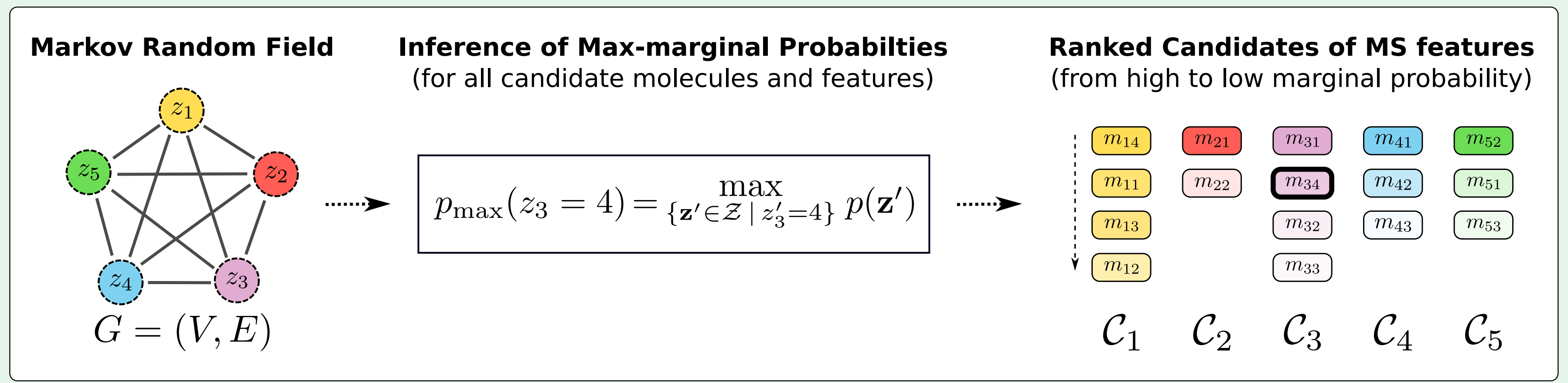

Markov Random Field
z_1
z_2
z_3
z_4
z_5
$G = (V, E)$
Inference of Max-marginal Probabilties
(for all candidate molecules and features)
$p_{\max}(z_3 = 4) = \max_{\{\mathbf{z}' \in \mathcal{Z} \,|\, z'_3 = 4\}} p(\mathbf{z}')$
Ranked Candidates of MS features
(from high to low marginal probability)
m_{14} m_{11} m_{13} m_{12}
m_{21} m_{22}
m_{31} m_{34} m_{32} m_{33}
m_{41} m_{42} m_{43}
m_{52} m_{51} m_{53}
$\mathcal{C}_1$ $\mathcal{C}_2$ $\mathcal{C}_3$ $\mathcal{C}_4$ $\mathcal{C}_5$

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 $\psi_i : \mathcal{Z}_i \rightarrow \mathbb{R}_{>0}$: $\psi_i(z_i = r) = f(x_i, m_{ir})$
  - f returns the MS matching score $\in (0, 1]$ of spectrum x_i and candidate m_{ir}
- Edge potential $\psi_{ij} : \mathcal{Z}_i \times \mathcal{Z}_j \rightarrow \mathbb{R}_{>0}$, 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

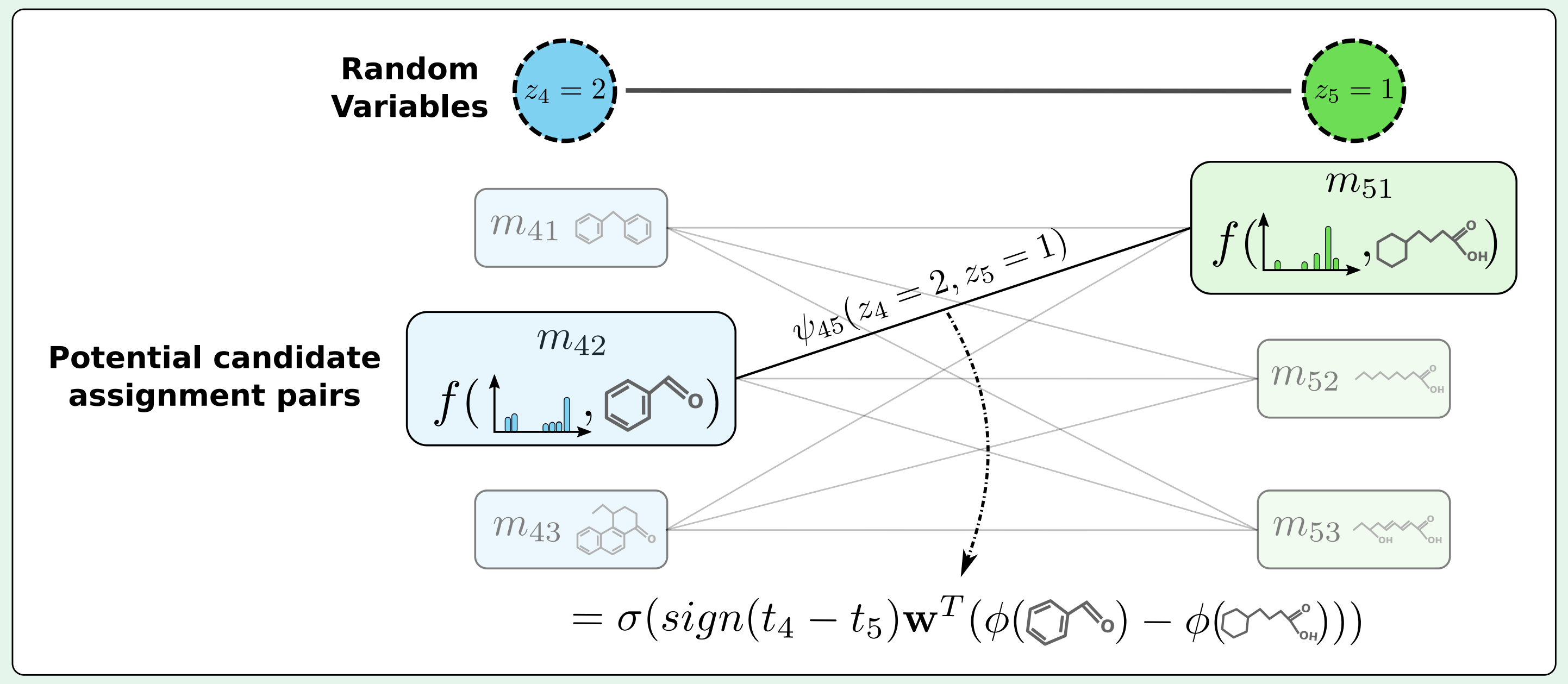

Random Variables
$z_4 = 2$
$z_5 = 1$
Potential candidate assignment pairs
m_{41}
m_{42}
$f($
m_{43}
m_{51}
m_{52}
m_{53}
O
OH
$\psi_{45}(z_4 = 2, z_5 = 1)$
$= \sigma(sign(t_4 - t_5)\mathbf{w}^T(\phi(\ldots) - \phi(\ldots)))$

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 (MS², RT)-tuples with each 310 candidates (median statistic)
  - 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
MS² + RT (Our)	21.3	52.9	64.0	74.3
MS² + RT (MetFrag & LogP)	20.5	49.1	61.2	72.6
Only MS² (baseline)	16.7	49.5	60.4	70.6

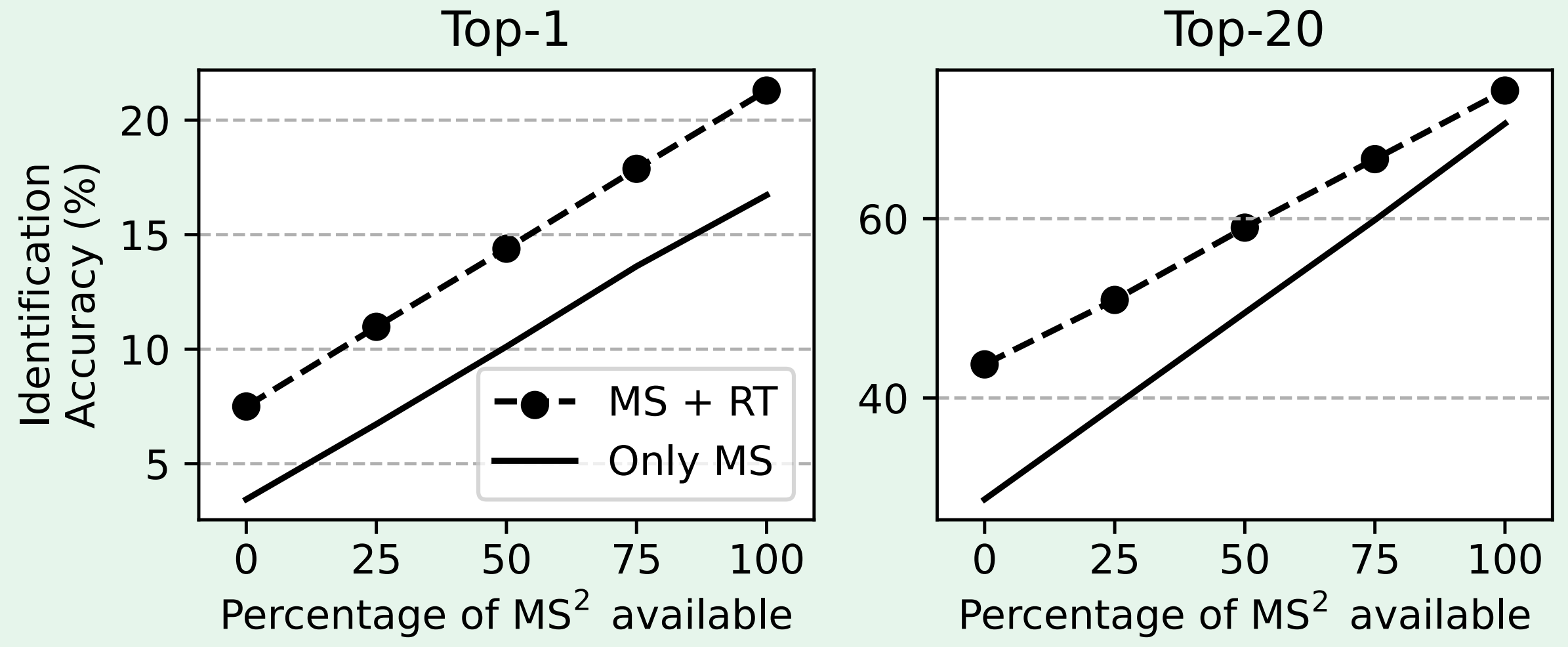
Experiment 2: Performance with different MS²-Scoring Methods

- MetFrag (in-silico fragmenter scores) and IOKR [3] as MS²-scoring methods

MS²-Scorer	Method	Top-1	Top-5	Top-10	Top-20
MetFrag	MS² + RT (our)	21.3	52.9	64.0	74.3
	Only MS² (baseline)	16.7	49.5	60.4	70.6
IOKR	MS² + RT (our)	26.7	52.1	62.5	70.3
	Only MS² (baseline)	25.1	49.5	60.3	67.6

Experiment 3: Missing MS² Spectra

- Simulating missing MS² information: Varying from 0% to 100% MS²
- If only MS¹: Use mass deviation between precursor and candidate molecule



References

[1] F. Aicheler, J. Li, M. Hoene, R. Lehmann, G. Xu, and O. Kohlbacher. Retention time prediction improves identification in nontargeted lipidomics approaches. *Analytical chemistry*, 2015.

[2] E. Bach, S. Szedmak, C. Brouard, S. Böcker, and J. Rousu. Liquid-chromatography retention order prediction for metabolite identification. *Bioinformatics*, 2018.

[3] C. Brouard, H. Shen, K. Dührkop, F. d'Alché-Buc, S. Böcker, and J. Rousu. Fast metabolite identification with Input Output Kernel Regression. *Bioinformatics*, 2016.

[4] K. Dührkop, M. Fleischauer, M. Ludwig, A. A. Aksenov, A. V. Melnik, M. Meusel, P. C. Dorrestein, J. Rousu, and S. Böcker. Sirius 4: a rapid tool for turning tandem mass spectra into metabolite structure information. *Nat Methods*, 2019.

[5] D. J. MacKay. *Information theory, inference and learning algorithms*. Cambridge university press, 2005.

[6] C. Ruttkies, E. L. Schymanski, S. Wolf, J. Hollender, and S. Neumann. Metfrag relaunched: incorporating strategies beyond in silico fragmentation. *Journal of Cheminformatics*, 2016.

[7] E. L. Schymanski, C. Ruttkies, M. Krauss, C. Brouard, T. Kind, K. Dührkop, F. Allen, A. Vaniya, D. Verdegem, S. Böcker, J. Rousu, H. Shen, H. Tsugawa, T. Sajed, O. Fiehn, B. Ghesquière, and S. Neumann. Critical assessment of small molecule identification 2016: automated methods. *Journal of Cheminformatics*, 2017.

[8] J. Stanstrup, S. Neumann, and U. Vrhovsek. Predret: Prediction of retention time by direct mapping between multiple chromatographic systems. *Analytical Chemistry*, 2015.

Acknowledgements: This work has been supported by the Academy of Finland grant 310107 (MACOME); and the Aalto Science-IT infrastructure. SR and JHW were supported by EPSRC (EP/R018634/1). This work started during a visit by JR to SR, funded through the Scottish Informatics and Computing Science Alliance (SICSA) distinguished visiting fellow scheme. **Contact (✉):** eric.bach@aalto.fi