Metabolite identification and molecular fingerprint prediction via machine learning

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Outline

1 Motivation
   - Metabolite identification
   - Mass spectrometry

2 Kernel framework
   - Mass kernels
   - Poisson-Binomial model

3 Experiments
   - SVM performance
   - Metabolite matching
Summary

- We present a “FingerID”\(^1\) machine learning framework for metabolite identification using tandem mass spectral data
  1. We introduce novel kernels for mass spectra for prediction of intermediate binary metabolite properties
  2. We introduce a statistical model to search metabolites with matching properties

\(^1\)sourceforge.net/p/fingerid
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Metabolomics bottlenecks

At the American Society for Mass Spectrometry (ASMS) conference 2009, a survey among the 600 participants asked [http://metabolomicssurvey.com]:

“From your perspective, what is the biggest bottleneck in metabolomics today?”

- Identification of metabolites: 35%
- Assigning biological significance: 22%
- Data processing or reduction: 14%
- Sample preparation: 8%
- Statistical analysis: 6%
- Validation or Utility studies: 5%
- Data acquisition or throughput: 3%
- Other: 2%
- No opinion: 6%
Metabolite identification

- Determination of the metabolic contents of the cell
- Requirement for further metabolomic analysis
- Mass spectrometry
  - Offers a “wide” view on the cell contents
  - Reveals only mass-to-charges (m/z), not structures
  - Average measurement error $\varepsilon$: true mass in range $[m - \varepsilon, m + \varepsilon]$

[Kind & Fiehn 2006: *Metabolomic database annotations via query of elemental compositions: mass accuracy is insufficient even at less than 1 ppm*]
Tandem mass spectrometry (MS/MS)

- Filter a single unknown compound by mass
  - Fragment the compound by high-energy collision into sub-structures called fragments
  - Measure the m/z of the fragments
- Each molecule produces a ‘unique’ set of fragments, and hence peaks
- The collision energy can be varied to produce more or less fragmented products
- ⇒ structural information

Data:
- The mass of the unknown metabolite (precursor mass)
- A list of (m/z,int) pairs of the fragments of the unknown metabolite
Current metabolite identification methods

Reference databases: Given an MS/MS spectrum of an unknown metabolite, search matching spectra from reference databases [Wiley, NIST, MassBank]

- Fails if the spectrum is not in the database, or if the measurement conditions/energies differ too much

Simulation: Simulate the fragmentation of candidate metabolites and match the observed spectrum against the simulated \textit{in silico} spectra

- MetFrag software: exhaustively cleave the bonds to produce possible fragments

Machine learning: Use the MS/MS peaks as a characterizing pattern to predict the structure of the metabolite

- No need for databases or simulation of the fragmentation process
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Machine learning problem

- Given a MS/MS spectrum measurement $\chi = \{x_1, \ldots, x_k\} \in \mathcal{X}$ as a collection of peaks $x = (mass, intensity)^T$ with average mass error $\varepsilon$, predict the measured unknown metabolite (a labeled graph) $M \in \mathcal{M}$

  $\Rightarrow$ A structured prediction problem from sets to graphs

  $$ f : \mathcal{X} \rightarrow \mathcal{M} $$

- We opt for a two-phase scheme instead

1. An intermediate prediction target: a vector of $m$ binary and independent structural properties (“fingerprints”) $y = (y_i)_{i=1}^m$, which characterizes the unknown metabolite structure

  $\Rightarrow$ A set of standard binary prediction problems (we use SVM’s)

  $$ f_i : \mathcal{X} \rightarrow \{0, 1\}^m \quad i = 1, \ldots, m $$

2. Reconstruct $M$ from fingerprints: We introduce a statistical model to find matching metabolite candidate’s based on the predicted property vector $\hat{y}$
Overview of the framework

![Chemical structure](image)

The workflow involves:

1. SVM: Training the model with molecular fingerprints.
2. Database matching: Searching the database for matching molecules.

**Molecule's fingerprints**
- **true**: 11000101...
- **pred**: 11100101...

**Selected peaks**
- m/z 73, 117.0, 145.1, 169.3, 187.4

**Intensity**
- Y-axis: 0 to 1
- X-axis: m/z 0 to 200

**Legend**
- **Unknown molecule** → **MS/MS** → **Spectrum**
## Fingerprints

- We use 528 structural fingerprints as a prediction targets
- Generated from OpenBabel’s FP3, FP4 and MACCS fingerprint sets
- The fingerprints should be predictable from MS/MS data, and be informative regarding the metabolite structure

### SMILES Interpretation

<table>
<thead>
<tr>
<th>SMILES</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>([N,n]~[C,c](~[O,o])~[N,n]',0)</code></td>
<td>( NC(O)N )</td>
</tr>
<tr>
<td><code>([N,n]~[C,c]([C,c])~[N,n]',0)</code></td>
<td>( NC(C)N )</td>
</tr>
<tr>
<td><code>([O,o]~[S,s]([O,o])~[O,o]',0)</code></td>
<td>( OS(O)O )</td>
</tr>
<tr>
<td><code>([C,c]-[O,o]',0)</code></td>
<td>( C-O )</td>
</tr>
<tr>
<td><code>([C,c]-[N,n]',0)</code></td>
<td>( C-N )</td>
</tr>
<tr>
<td><code>[+]</code></td>
<td>cation</td>
</tr>
<tr>
<td><code>[CX3H1](=O)[\#6]</code></td>
<td>aldehyde</td>
</tr>
<tr>
<td><code>[#6][CX3](=O)[\#6]</code></td>
<td>ketone</td>
</tr>
<tr>
<td><code>[#6][CX3]([SX1])[\#6]</code></td>
<td>Thioketone</td>
</tr>
<tr>
<td><code>[SX2H][c]</code></td>
<td>Arylthiol</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Mass spectral kernels

- We introduce kernels for mass spectral data \( \chi = \{x_1, \ldots, x_k\} \)
- We extract three classes of features from MS/MS spectra into sparse vectors with ‘bins’ of fixed width of 1

\[
\phi_{\text{peaks}}(\chi)_i = \sum_{(\text{mass}, \text{int}) \in \chi} \delta_i \pm 0.5(\text{mass}) \cdot \text{int} \\
\phi_{\text{nloss}}(\chi)_i = \sum_{(\text{mass}, \text{int}) \in \chi} \delta_i \pm 0.5(\text{prec}(\chi) - \text{mass}) \cdot \text{int} \\
\phi_{\text{diff}}(\chi)_i = \sum_{(\text{mass}, \text{int}) \in \chi} \delta_i \pm 0.5(|\text{mass} - \text{mass}'|) \cdot \text{int} \cdot \text{int}'
\]

where \( \delta \) is an indicator function

\( \phi_{\text{peaks}}(\chi)_{73} = 0.04^* \)
\( \phi_{\text{nloss}}(\chi)_{18} = 0.11^{**} \)
\( \phi_{\text{diff}}(\chi)_{28} = 1.0 \times 0.90 = 0.90^{***} \)
The integral mass kernels are

\[
K_{peaks}(\chi, \chi') = \langle \phi_{peaks}(\chi, \chi') \rangle
\]

\[
K_{nloss}(\chi, \chi') = \langle \phi_{nloss}(\chi, \chi') \rangle
\]

\[
K_{diff}(\chi, \chi') = \langle \phi_{diff}(\chi, \chi') \rangle
\]

A summed kernel

\[
K_{full} = K_{peaks} + K_{nloss} + K_{diff}
\]

correspond to a concatenation of the feature sets

\[
[\phi_{peaks}; \phi_{nloss}; \phi_{diff}].
\]

An explicit feature mapping \( \phi : \mathcal{X} \rightarrow \mathbb{R}^D \)

An alignment problem: does a peak 70.493m/z belong to bin 70 or 71 with mass error \( \varepsilon = 0.5 \)?
Spectral density model

- We incorporate the mass measurement error directly into the features
- We model each peak as a 2-dimensional gaussian

\[ p(x) \sim \mathcal{N}(x, \Sigma). \]

The spectrum becomes a gaussian mixture model

\[ p(\chi) = \frac{1}{k} \sum_{i=1}^{k} \mathcal{N}(x_i, \Sigma) \]

The \( \Sigma = \begin{bmatrix} \sigma_{mass} & 0 \\ 0 & \sigma_{int} \end{bmatrix} \) models the error
High resolution probability product kernel

- Kernels between sets or distributions [Jebara & Kondor 2004]
- Represent a spectrum $\chi = \{x_1, \ldots, x_k\}$ of peaks with a probability distribution $p(\chi)$
- The kernel $K(\chi, \chi') \equiv K(p, p')$ is then a similarity between probability distributions as the integral of the product distribution:

$$K(p, p') = \int_{\mathbb{R}^2} p(x)p'(x)dx$$

- Interpretation as expectation of one distribution under the other (expectation likelihood kernel):

$$\int_{\mathbb{R}^2} p(x)p'(x)dx = \mathbb{E}_p[p'(x)] = \mathbb{E}_{p'}[p(x)]$$

- Feature map: $\varphi : \chi \rightarrow p(\chi)$, the kernel $K(p, p') = \langle p, p' \rangle$ in $\ell_2$ space
- Closed form solution for gaussian mixtures (fast)
- We use the probability product kernel over the three features
Fingerprints into metabolites

- We predict the fingerprint vector $\hat{y}$ of the unknown metabolite using SVM’s and the mass spectral kernels.
- Next, we find candidate metabolites with matching fingerprints from molecular databases (PubChem).
- The fingerprint predictions contain almost always errors and thus the candidate metabolite with exactly matching fingerprints is rarely correct.
  - We list candidates according to how confident we are in specific predictions.
  - The cross-validation prediction accuracies $(p_i)_{i=1}^m$ of a fingerprint $i$ being correctly predicted are used to determine which fingerprints we allow to mismatch.
Poisson-Binomial model

- Poisson-Binomial model for a particular fingerprint vector $y$ being true given the prediction $\hat{y}$ and the prediction accuracies $p = (p_i)_{i=1}^m$:

$$ P(y|p, \hat{y}) = \prod_{i=1}^m p_{[y_i = \hat{y}_i]} (1 - p_i)_{[y_i \neq \hat{y}_i]} $$

- Maximum value at $y = \hat{y}$
- A high $p_i$ indicates that a candidate with non-matching $i$’th fingerprint is unlikely to be true
- A low $p_i$ indicates that a candidate with non-matching $i$’th fingerprint might be true

- Each candidate metabolite gets a score based on its fingerprint vector:

$$ score(M) = P(y(M)|p, \hat{y}) $$

- We rank metabolites by score (success = true metabolite in top10)
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Experiments

- Three datasets from MassBank
  - ‘QqQ’ \( (n = 514, m = 286) \): A low-accuracy Quadrupole dataset with repeated measurements at collision energies 10eV, 20eV, ..., 50eV
  - ‘Ltq’ \( (n = 293, m = 128) \): A high-accuracy LTQ Orbitrap dataset
  - ‘Lipids’ \( (n = 403, m = 20) \): A high-accuracy LTQ Orbitrap dataset of non-common phosphatidylethanolamines

- Standard SVM’s, 5-fold crossvalidation, C parameter from \( \{10^0, \ldots, 10^4\} \)

- Candidate metabolites are queried from
  - KEGG (a small database of over 14,000 metabolites)
  - PubChem (a large general-purpose repository of over 30 million molecules)

1. We evaluate the accuracy of fingerprint prediction using different kernels
2. We evaluate the ranks of true metabolites using fingerprint predictions
### Finger print prediction accuracy

<table>
<thead>
<tr>
<th>Kernel</th>
<th>Single spectra (CE eV)</th>
<th>Multiple spectra</th>
<th>Ltq</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 20 30 40 50 K_e merge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Integral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{p}$, linear quadr.</td>
<td>87.8 88.2 88.8 89.3 89.5</td>
<td>89.5 89.2</td>
<td>85.5</td>
<td>98.4</td>
</tr>
<tr>
<td>$K_{nl}$</td>
<td>87.9 88.3 88.8 89.4 89.6</td>
<td>89.9 89.8</td>
<td>84.4</td>
<td>98.1</td>
</tr>
<tr>
<td>$K_{df}$</td>
<td>88.4 88.8 88.8 88.7 89.2</td>
<td>89.4 89.0</td>
<td>86.3</td>
<td>98.8</td>
</tr>
<tr>
<td>$K_{p+nl}$</td>
<td>87.8 88.0 87.7 87.8 88.2</td>
<td>89.6 89.3</td>
<td>86.1</td>
<td>98.7</td>
</tr>
<tr>
<td>$K_{p+df}$</td>
<td>87.8 88.0 87.8 87.9 88.3</td>
<td>88.0 87.9</td>
<td>82.6</td>
<td>97.1</td>
</tr>
<tr>
<td>$K_{p+nl+df}$</td>
<td>87.8 88.0 87.8 87.9 88.3</td>
<td>87.9 87.9</td>
<td>82.9</td>
<td>96.9</td>
</tr>
<tr>
<td><strong>High resolution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{p}^\phi$</td>
<td>88.0 88.6 89.1 89.1 89.4</td>
<td>89.3 89.4</td>
<td>86.7</td>
<td>98.6</td>
</tr>
<tr>
<td>$K_{nl}^\phi$</td>
<td>88.2 89.1 89.5 89.7 89.9</td>
<td>89.3 90.0</td>
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<td>89.8 89.6</td>
<td>88.8</td>
<td>99.1</td>
</tr>
<tr>
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<td>89.0 89.8 89.7 89.5 89.6</td>
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<td>89.2 89.3</td>
<td>83.7</td>
<td>97.8</td>
</tr>
<tr>
<td>$K_{p+nl+df}^\phi$</td>
<td>88.6 89.0 88.9 88.6 88.6</td>
<td>89.2 89.5</td>
<td>83.9</td>
<td>97.1</td>
</tr>
<tr>
<td><strong>Random</strong></td>
<td>89.0 89.9 90.1 90.1 90.2</td>
<td>90.5 90.5</td>
<td>91.1</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>89.2 90.1 90.3 90.3 90.4</td>
<td>90.1 90.8</td>
<td>89.6</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>88.8 89.4 89.5 89.5 89.5</td>
<td>90.0 90.0</td>
<td>86.5</td>
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<tr>
<td></td>
<td>88.9 89.5 89.7 89.8 89.8</td>
<td>89.8 90.4</td>
<td>84.9</td>
<td>97.5</td>
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<tr>
<td></td>
<td>89.1 90.0 90.3 90.2 90.2</td>
<td>90.6 90.7</td>
<td>90.5</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td><strong>89.2 90.1 90.4 90.5 90.4</strong></td>
<td><strong>90.2 91.1</strong></td>
<td>88.6</td>
<td>98.0</td>
</tr>
</tbody>
</table>

| random          | 87.3 87.2 87.2 87.2 87.7 | 87.3 87.7 | 88.3 |     |

**Table**: The classification accuracies (in %) of the three datasets with various kernels. Abbreviations: $p$ is peaks, $nl$ is neutral loss, and $df$ is difference kernel.
Figure: Scatter plot of the aggregate average accuracy/F$_1$ across the three datasets with different kernel features. The open markers represent higher accuracy/F$_1$ ratio in a linear kernel.
Individual fingerprint prediction accuracies

Figure: SVM prediction accuracies of individual fingerprints of the LTQ dataset with high resolution and integral mass kernels. The bottom of the bars is the baseline classifier.
**Ranks**

*Figure*: The ranks of the true metabolite according to the high resolution kernel and the Poisson-Binomial matching model with three datasets and two molecular repositories.
Comparison to MetFrag

- MetFrag is a state-of-the-art computational metabolite identification package\(^2\).
- MetFrag simulates the fragmentation process and tries to match the simulated spectra against the observed.
- MetFrag also extracts candidate metabolites from KEGG or PubChem.

<table>
<thead>
<tr>
<th>Molecular database</th>
<th>Spectral dataset</th>
<th>FingerID match</th>
<th>Avg. rank</th>
<th>rank (\leq) 10</th>
<th>MetFrag match</th>
<th>Avg. rank</th>
<th>rank (\leq) 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kegg</td>
<td>QqQ</td>
<td>17</td>
<td>3.2</td>
<td>16/17</td>
<td>16</td>
<td>5.1</td>
<td>9/16</td>
</tr>
<tr>
<td></td>
<td>Ltq</td>
<td>20</td>
<td>3.8</td>
<td>18/20</td>
<td>12</td>
<td>5.6</td>
<td>11/12</td>
</tr>
<tr>
<td>PubChem</td>
<td>QqQ</td>
<td>11</td>
<td>905</td>
<td>8/11</td>
<td>2</td>
<td>68</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>Ltq</td>
<td>20</td>
<td>58</td>
<td>9/20</td>
<td>1</td>
<td>20</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Table: Comparison of metabolite identification against MetFrag on a subset of 20 spectra from both ‘QqQ’ and ‘Ltq’, respectively.

\(^2\)Wolf, Schmidt, Muller-Heinemann & Neumann 2010; [msbi.ipb-halle.de/MetFrag/](http://msbi.ipb-halle.de/MetFrag/)
Conclusions

- **Software FingerID**: [sourceforge.net/p/fingerid](http://sourceforge.net/p/fingerid)
- A machine learning framework for metabolite identification
- Probability product kernels provide a flexible model for mass spectra
- Future work: explore structured prediction, feature selection (L1)
Thank you

Thank you!