CFM-ID: A Web Server for Annotation, Spectrum Prediction and Metabolite Identification from MS/MS

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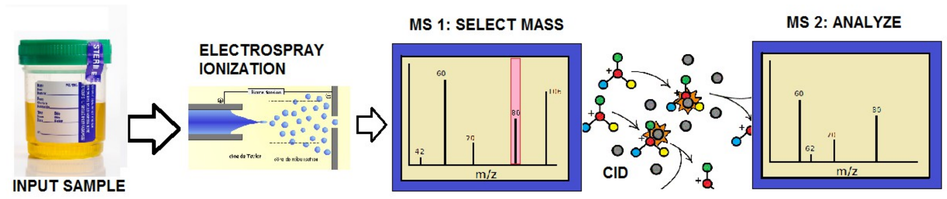
Summary

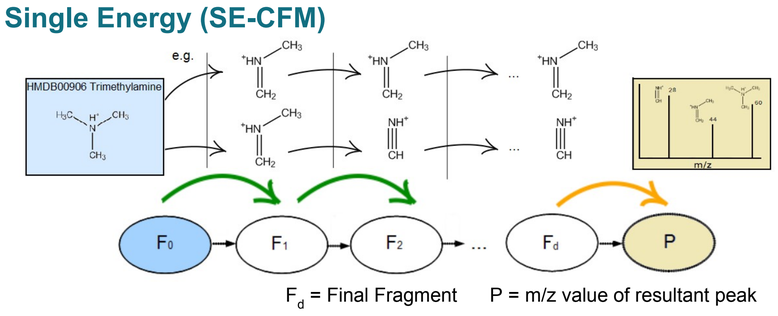
- **Goal:** Automated identification of metabolites from tandem mass spectra (MS/MS).
- **Existing Methods:**
  - Search against reference databases of measured spectra [3,4,5] – but limited coverage!
  - Enumerate all ways molecules could break[6,10], and/or make a heuristic selection of likely breaks[6,11,12] to predict spectra – usually predict far more peaks than actually occur.
- **Our approach:**
  - Design Competitive Fragmentation Modeling (CFM)[2], a model for Electrospray (ESI) MS/MS fragmentation. Derive parameters for CFM from MS/MS data.
- **CFM-ID:** A web server that uses CFM to provide three utilities associated with interpretation of MS/MS spectra:
  - Spectrum Prediction, Peak Assignment and Compound Identification.
- **Experimental Results:**
  - Spectrum Prediction: Better Jaccard scores vs full enumeration of possible peaks.
  - Compound Identification: Better ranking results vs existing methods MetFrag [6] and FingerID[7] querying KEGG[8] and PubChem[9] for possible candidates.

Competitive Fragmentation Modeling (CFM) [2]



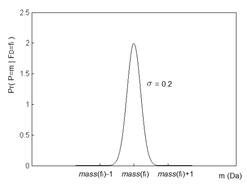
- Model ESI-MS/MS (above) fragmentation as a stochastic, homogeneous, Markov process of state transitions between charged fragments (below).

Single Energy (SE-CFM)



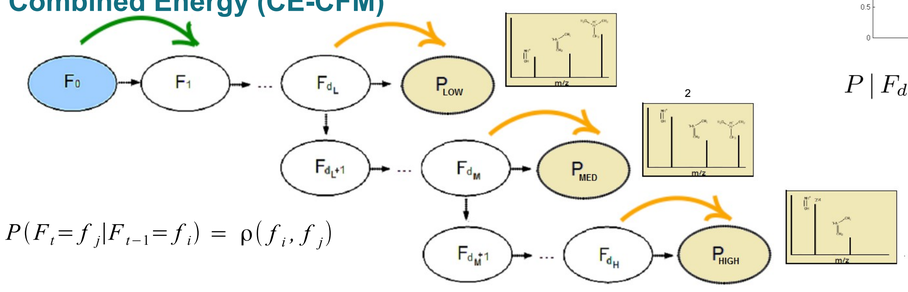
F_d = Final Fragment P = m/z value of resultant peak

- Observation model links F_d to P via Gaussian distribution.



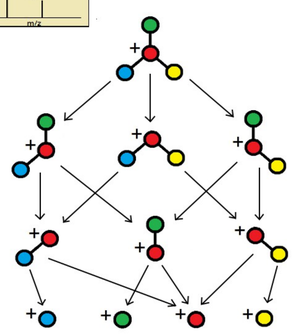
$$P \mid F_d \sim \mathcal{N}(mass(F_d), \sigma)$$

Combined Energy (CE-CFM)



$$P(F_t = f_j \mid F_{t-1} = f_i) = \rho(f_i, f_j)$$

- The initial molecule (F_0) and the output peak (P) are observed.
- All intermediate fragments (F_1..F_d) are latent.
- Possible transitions: Enumerate a graph of all possible fragmentations for each molecule (right), similar to [6,10].

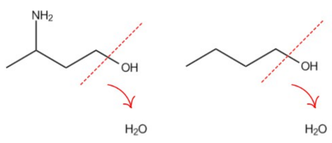


- Softmax transition function is competitive:

→ a particular break is likely to occur only if no other breaks are substantially more likely.

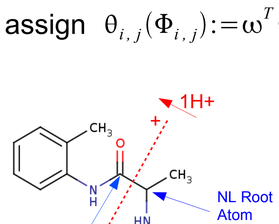
$$\rho(f_i, f_j) = \begin{cases} \frac{\exp \theta_{i,j}}{1 + \sum_k \exp \theta_{i,k}} & : f_i \neq f_j \text{ and } f_i \rightarrow f_j \text{ is possible} \\ \frac{1}{1 + \sum_k \exp \theta_{i,k}} & : f_i = f_j \\ 0 & : f_i \rightarrow f_j \text{ is not possible} \end{cases}$$

e.g. the NH_2 group in the left molecule reduces the likelihood of the H_20 loss.



- Given $\Phi_{i,j}$ = chemical features associated with break (f_i,f_j), assign $\theta_{i,j}(\Phi_{i,j}) := \omega^T \Phi_{i,j}$.

e.g. $\Phi_{i,j}$
- Break Pair:
  - C-C? true, C-N? false...etc
- Root paths (length 2 and 3):
  - C-N on ion side? true ...
- Gasteiger Charges of root atoms
- Hydrogen Movement
  - 1H+ moves from NL to Ion
- Ring Break Features
  - Size of ring, broken bond distance, aromatic ...

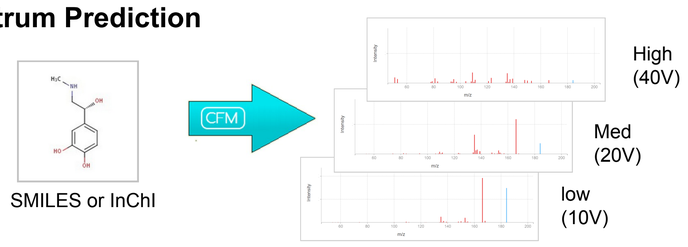


- Set model parameters ω using a maximum likelihood approach applied to a training set using the Expectation Maximization (EM) algorithm.

CFM-ID Web Server [1]

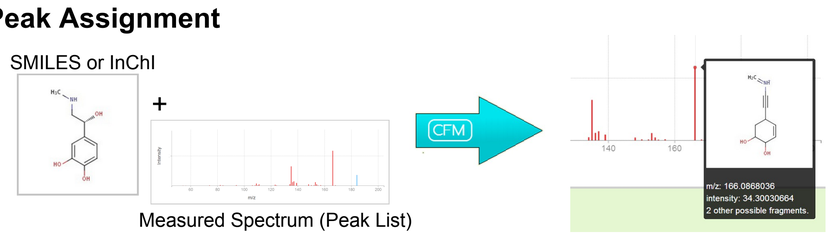
- Supports three sub-tasks for automated metabolite identification from MS/MS data:

Spectrum Prediction



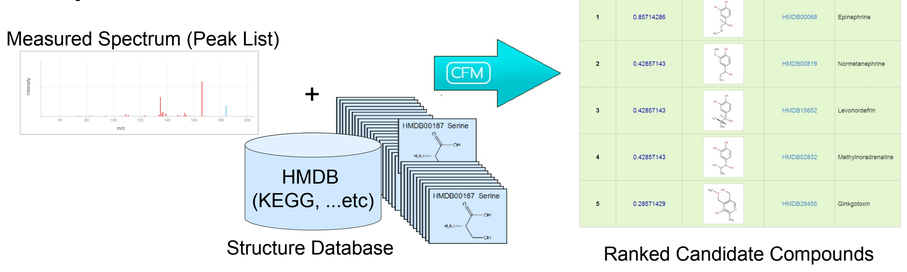
- Runs trained CFM model forward to predict spectra for low, medium and high collision energies.

Peak Assignment



- Assigns fragments within mass tolerance of each peak.
- Orders fragments according to CFM likelihoods.

Compound Identification



- Predicts spectra for all candidate compounds.
- Ranks compounds by Jaccard Score between measured and predicted spectra.

- Over 300,000 precomputed spectra for compounds in HMDB[5] and KEGG[8]!!

Available free at http://cfmid.wishartlab.com

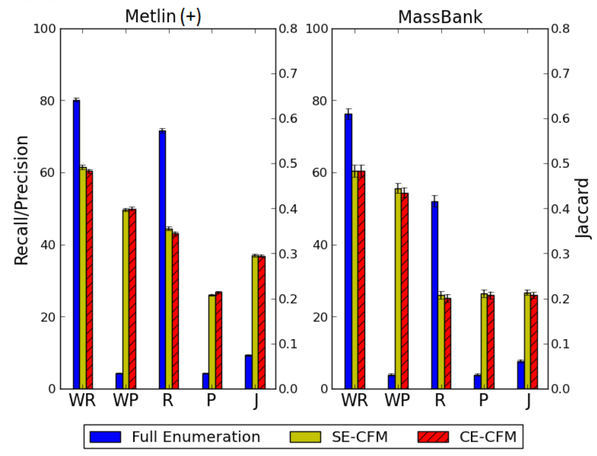
Experimental Validation

Data Sets:

Data Set	# Mols	Mode	Device	Energies
Metlin (+)	1491	+	Agilent 6510 Q-TOF	10V, 20V, 40V
MassBank	192	+	Agilent 6520 Q-TOF	10V, 20V, 40V
HMDB	500	+	Quattro QqQ	10V, 25V, 40V
Metlin (-)	976	-	Agilent 6510 Q-TOF	10V, 20V, 40V

- Metlin[3] tests used a 10-fold cross validation framework. MassBank[4] and HMDB[5] tests used a model trained on the Metlin data.

Spectrum Prediction:
- Compare vs full enumeration of all possible fragments (right).
- Low energy (10V) spectra better predicted.
- Positive though imperfect correlation between measured and predicted intensity values - Pearson correlations of 0.7 (10V), 0.6 (20V) and 0.45 (40V).



(R)ecall: Percent of measured peaks predicted.
(P)recision: Percent of predicted peaks measured.
(WR) and (WP): Recall and Precision weighted by peak intensity.
(J)accard: Predicted (A) vs measured (B) peaks | A ∩ B | / | A U B|

Compound Identification:
- Query KEGG[8] and PubChem[9] for candidates within tolerance of the known mass of the target.
- Compare against other methods (below).

	Data Set	Querying KEGG (±0.5 Da, # cand. ≈ 22) R = 1	R ≤ 5	MF = 1	Querying PubChem (±5 ppm, # cand. ≈ 1025) R = 1	R ≤ 10	MF = 1
CFM-ID	Metlin (+)	76.5	96.2	94.8	10.9	40.7	89.3
	MassBank	72.8	97.5	97.5	7.3	46.9	93.2
	HMDB	23.1	58.1	39.0	4.1	24.9	88.4
	Metlin (-)	72.1	96.5	95.2	13.4	51.4	93.8
MetFrag	Metlin (+)	51.9	89.9	72.2	5.7	30.5	82.6
	MassBank	48.1	88.9	71.6	4.7	20.8	85.4
	HMDB	13.3	43.6	28.3	2.6	13.4	88.0
	Metlin (-)	44.7	80.7	62.3	7.5	28.8	81.8
FingerID	Metlin (+)	8.7	36.1	17.0	1.3	9.3	67.7
	MassBank	14.8	37.0	19.8	0.5	5.7	71.9

Values are % of data set (restricted to those with correct structure in queried candidate list).
R : Ranking of the correct molecule in the candidate list
MF : Ranking of the correct molecular formula
cand. ≈ N : The median number of molecules in the candidate list

- **MetFrag**[6]: Combinatorial method with heuristics to rank likely breaks.
- **FingerID**[7]: uses SVMs to predict each bit of a molecular fingerprint, then searches using that fingerprint.

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