Random Weighted Strings and Weighted HMMs: Computation of Cleavage Fragment Statistics in Mass Spectrometry

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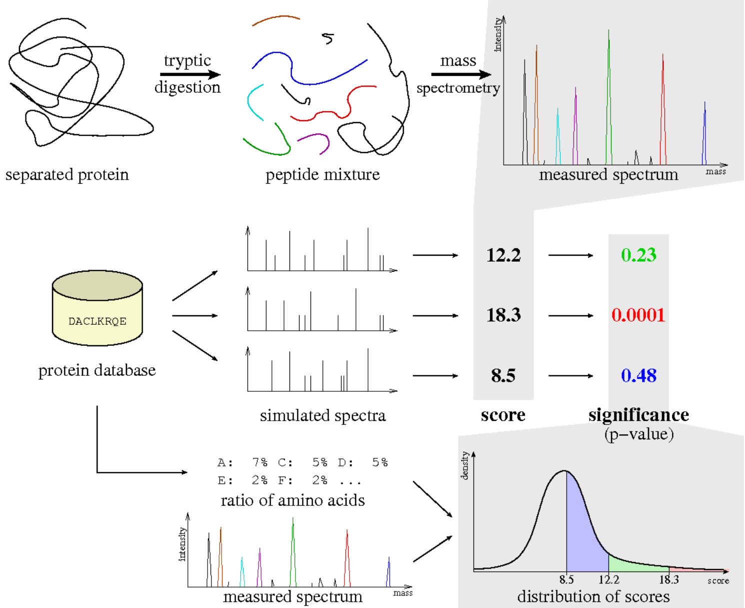
Peptide Mass Fingerprinting

Protein Identification

- Isolate all copies of one protein from a cell
- Digest these proteins deterministically into fragments (peptides)
- Measure fragment masses by mass spectrometry
- Compare peptide mass fingerprint (PMF) to predicted PMF of database proteins
- Return database protein that “fits best”
- Compute significance of “best fit”

Peptide Mass Fingerprinting



separated protein

tryptic digestion

peptide mixture

mass spectrometry

measured spectrum

protein database

simulated spectra

score

significance (p–value)

12.2 → 0.23

18.3 → 0.0001

8.5 → 0.48

ratio of amino acids

```
A:  7% C:  5% D:  5%
E:  2% F:  2% ...
```

measured spectrum

distribution of scores

Protein Space – just strings

Definition (Protein sequence)

A protein is a word of some length $\ell \geq 1$ over amino acid alphabet Σ.

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For a given length $\ell \geq 1$ and amino acid frequencies $f = (f(a))_{a \in \Sigma}$, assign a probability to every protein sequence $s = (s_1, \ldots, s_\ell)$:

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No masses so far

Protein masses – weighted strings

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Every amino acid a has a mass distribution $\mathcal{L}_a$, derived from

- isotopic distributions of its component atoms,
- modification probabilities,
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Definition (Protein mass)

Every amino acid s_i of protein $s \in \Sigma^\ell$ has a random mass μ_{s_i} drawn from its distribution $\mathcal{L}_{s_i}$.

$$\mu_s = \mu_{s_1} + \mu_{s_2} + \cdots + \mu_{s_\ell} \quad \text{and} \quad \mathcal{L}_s = \mathcal{L}_{s_1} \star \mathcal{L}_{s_2} \star \cdots \star \mathcal{L}_{s_\ell}.$$

Protein Cleavage – getting the PMF

Definition (Standard cleavage scheme)

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$\Gamma = \{\mathtt{K}, \mathtt{R}\}$, $\Pi = \{\mathtt{P}\}$; cuts after lys or arg unless followed by pro.
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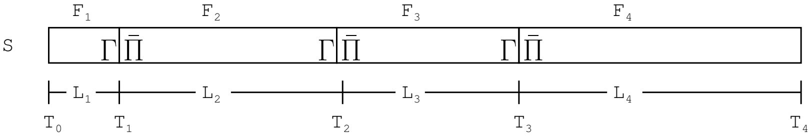

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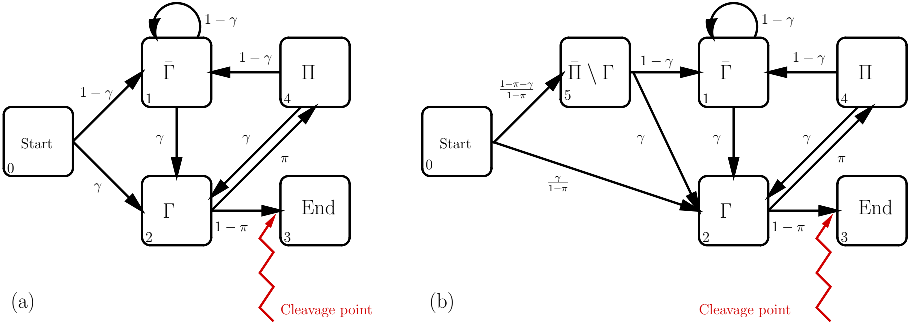
Is there an exact and efficient method?

"Weighted HMMs" (wHMMs), or "Mass-accumulating Markov Chains"



wHMM: generative probabilistic cleavage model

Left: Initial fragment. Right: Following fragments.

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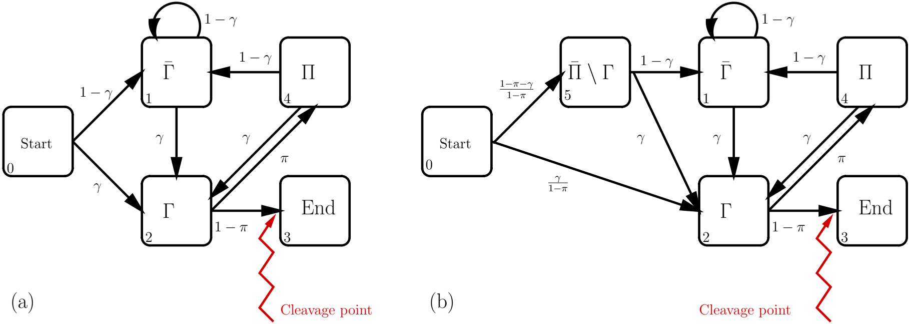
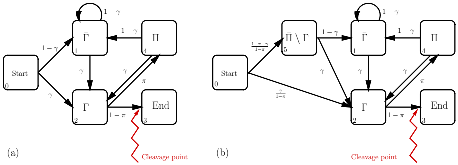


wHMM: generative probabilistic cleavage model
Left: Initial fragment. Right: Following fragments.

A wHMM can be derived from a standard cleavage scheme (Γ, Π), or from more complicated cleavage rules.

Using wHMMs for Probability Computations



- $h_i^l[m] := \mathbb{P}(\text{in state } i \text{ after } l \text{ steps, accumulated mass } m)$,
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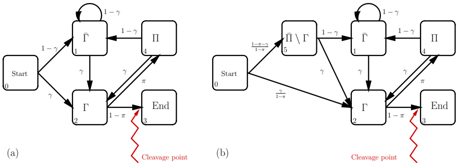

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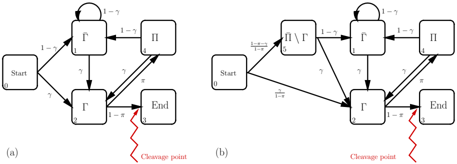

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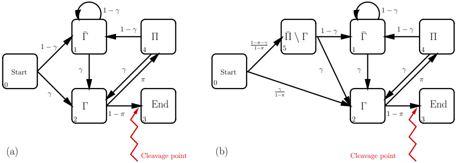

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$$\mathbb{P}(\text{fragment has length } l \text{ and mass } m) = h^{l+1}_{\text{"End"}}[m]$$

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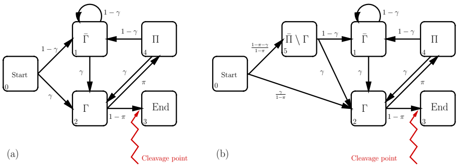

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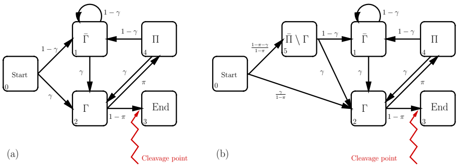

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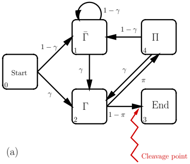

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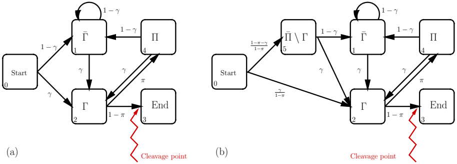

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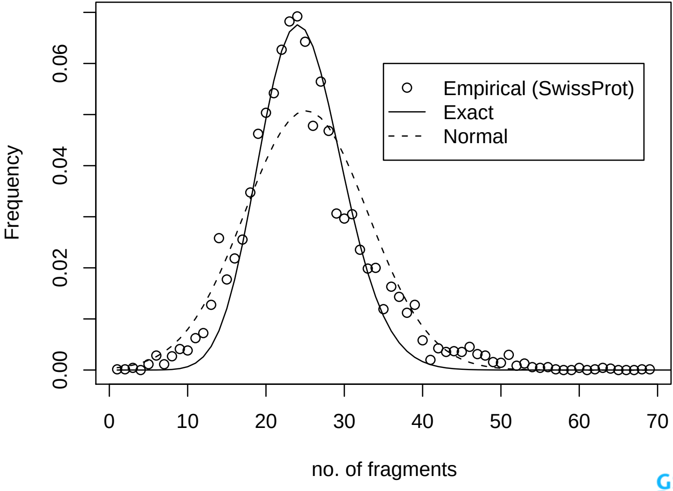
This is an update formula for the mass-state distribution.

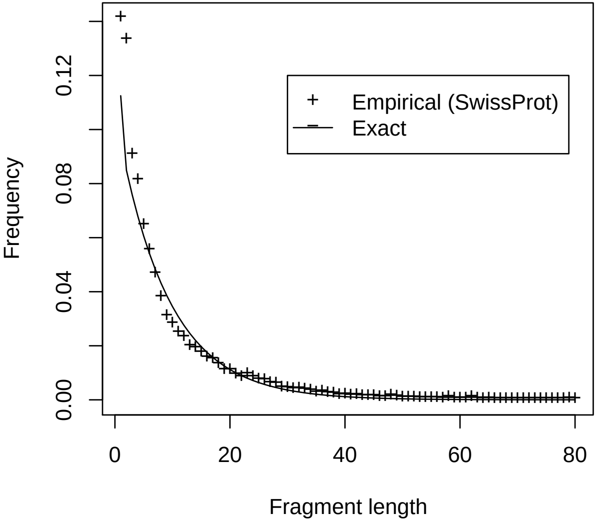
Results: Number of Fragments

Fragment number distribution of proteins of length 207 ± 7.



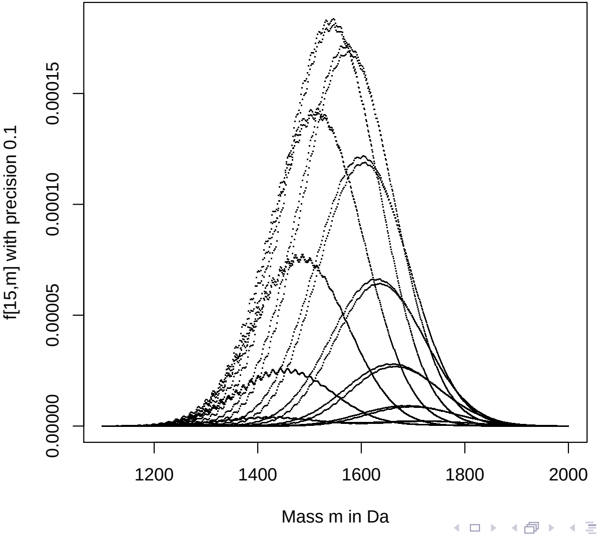
Fragment Lengths

Distribution of fragment lengths of SwissProt proteins



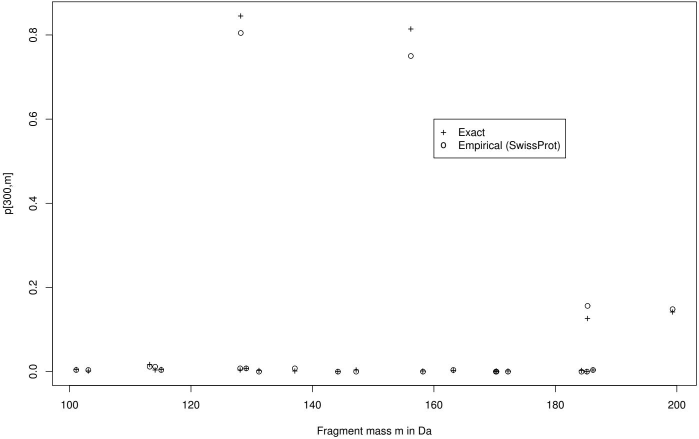
Joint Length-Mass Distribution

Fragment mass distribution; length = 15, High precision = 0.1 Da.



Mass Occurrence Probabilities

Fragment mass occurrence probabilities for proteins of length 300



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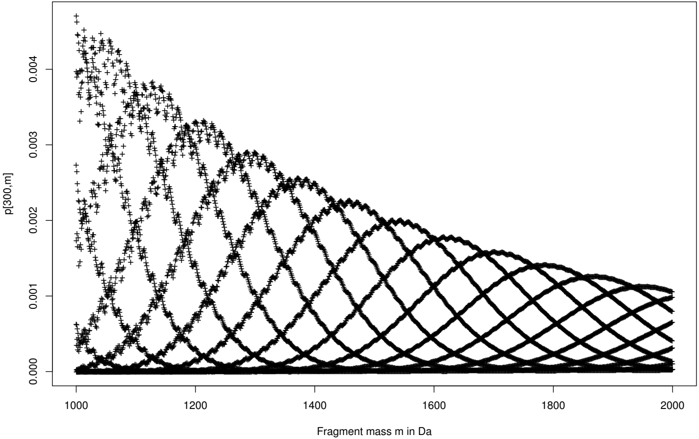

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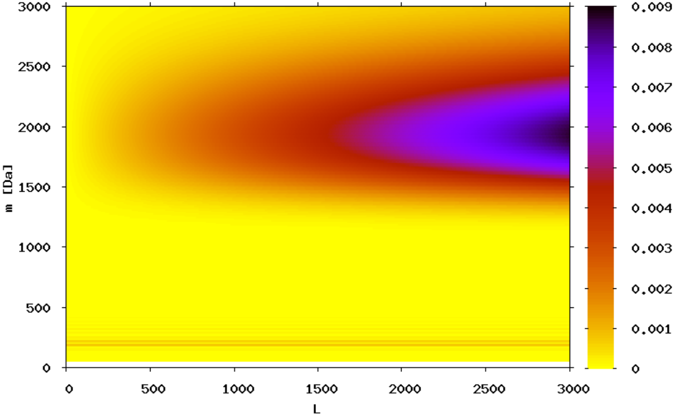

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- New computational framework "wHMM"
- Only aa frequencies needed
- Elegant formulation and update equation:
  $H^{(l)} = (H^{(l-1)} \cdot P) \star G.$
- Applicable to probability computations in mass spectrometry, to significance computations for peptide mass fingerprinting, e.g., what's the probability that a random protein contains a fragment with mass in a given range?

Acknowledgments

Joint work with

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Thank you for listening

Questions?