A Model for Enzymatically ^{18}O-Labeled Mass Spectra

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Outline

Mass spectrometry

- Allows to separate (peptide) molecules by their atomic mass
- Allows to quantify the abundance of the molecules in a sample

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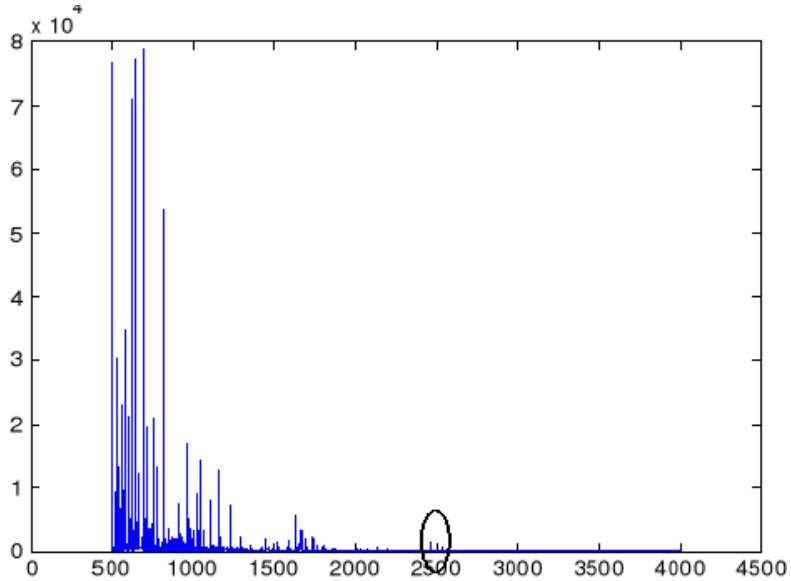

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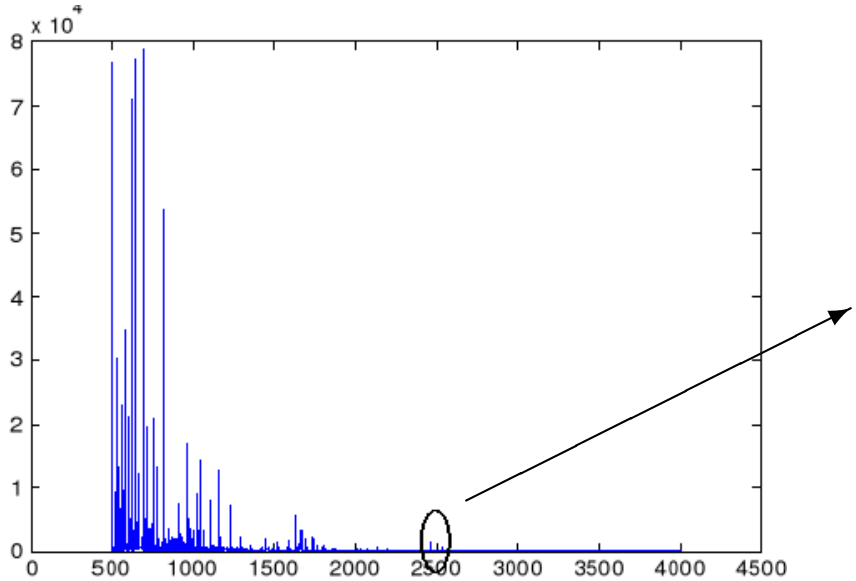

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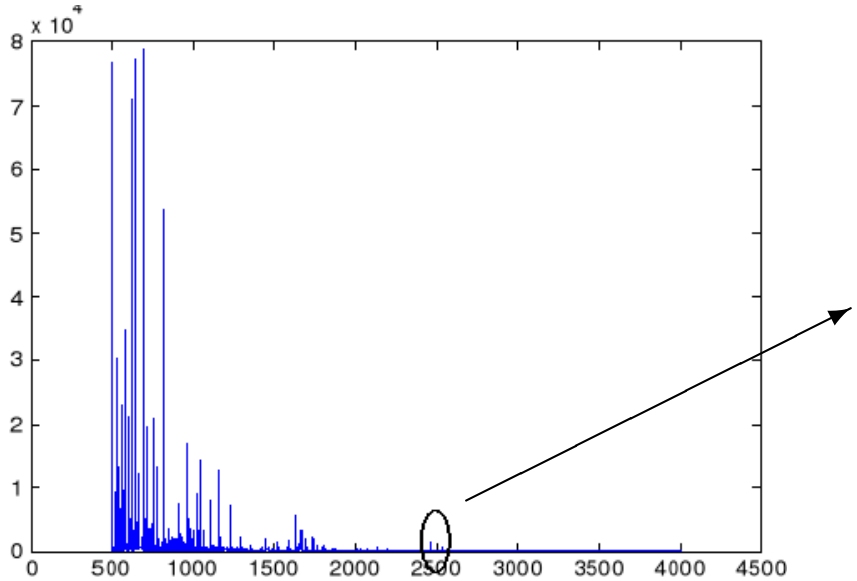
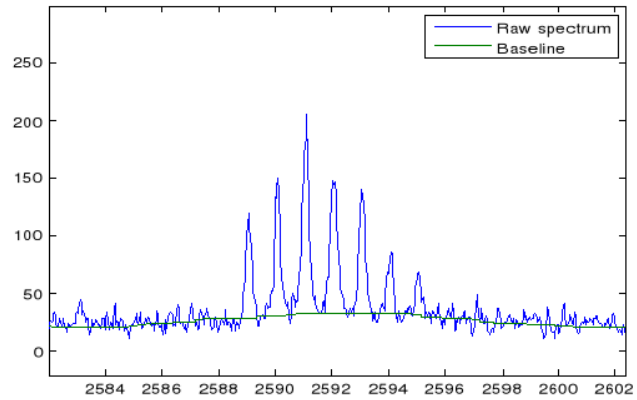




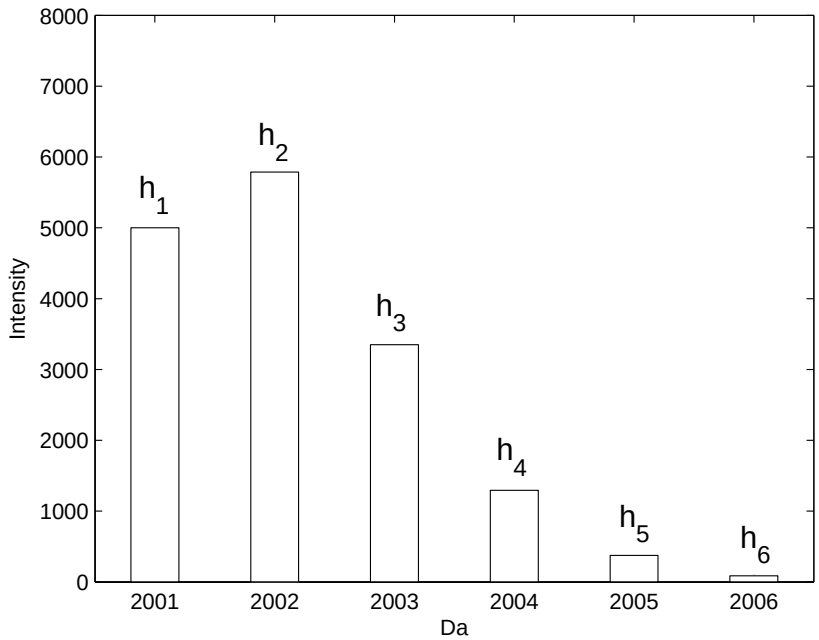
Isotopic distribution

- Molecules of a peptide contain different isotopes of chemical elements:
  $(^{1}H,^{2}H)$ $(^{16}O,^{17}O,^{18}O)$ $(^{12}C,^{13}C)$ $(^{14}N,^{15}N)$ $(^{32}S,^{33}S,^{34}S,^{36}S)$
- Lead to isotopic variants of a molecule, with masses differing by ≈ 1 Da

- Distribution represented by *isotopic ratios*:

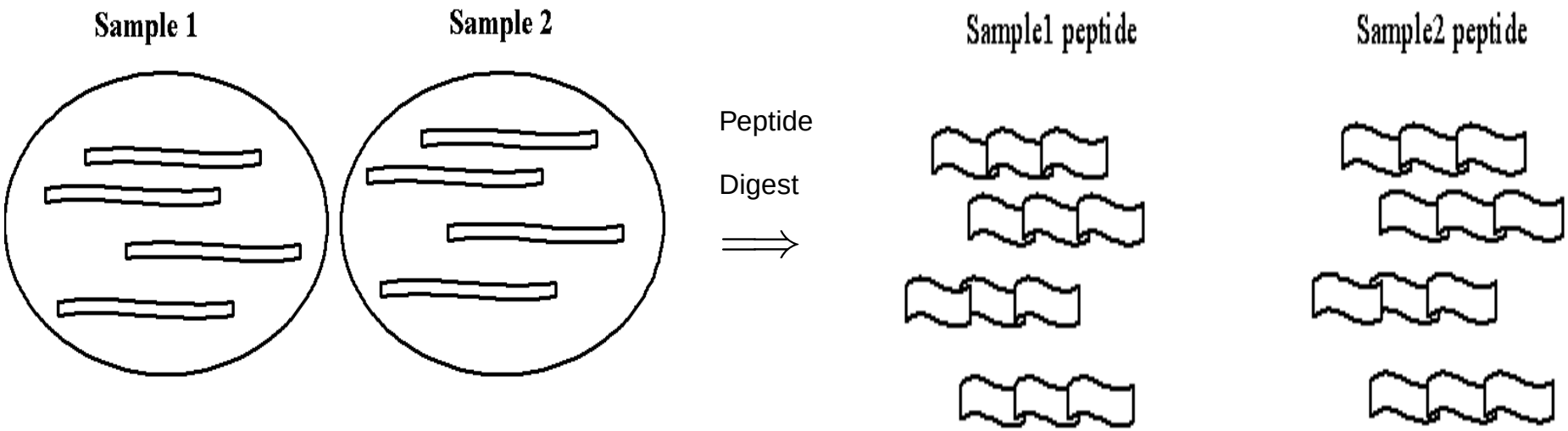
  $R_1 = h_1/h_1,$
  $R_2 = h_2/h_1,$
  $R_3 = h_3/h_1,$

  $\ldots$



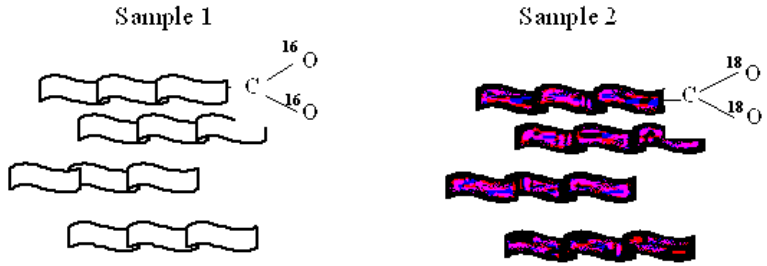
^{18}O-labeling strategy

- Reduces the beween-spectra variability by comparing samples in the same spectrum
- Idea similar to double-channel cDNA microarrays



^{18}O-labeling strategy

- Samples get labeled ($^{16}O \longrightarrow {}^{18}O$)



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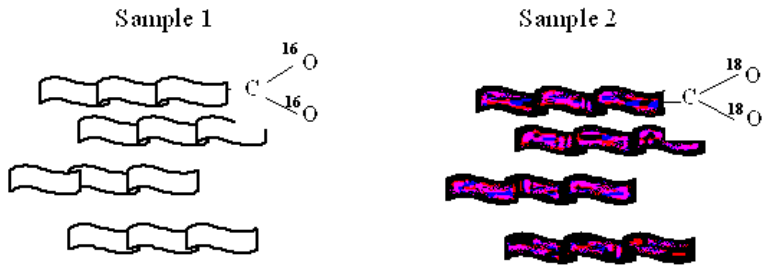
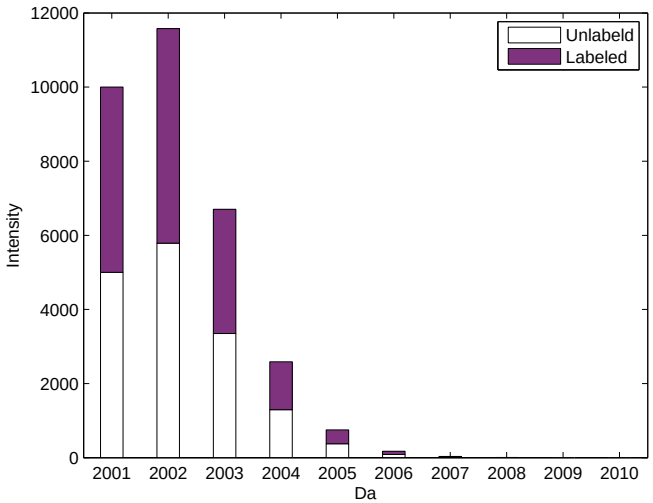


- Labeled samples are processed simultaneously



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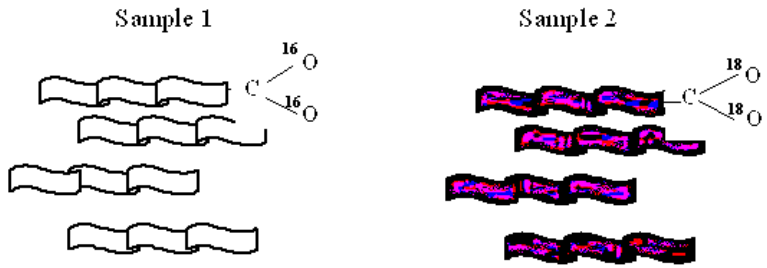

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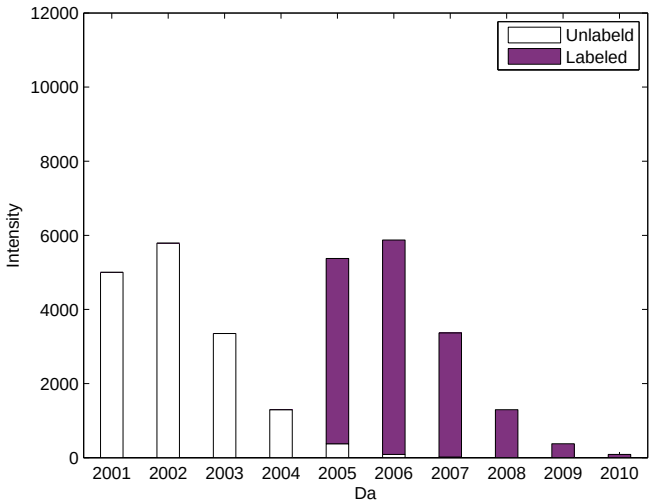

- Ideally, the labeled sample shifted by 4 Da ($2 \times {}^{18}O$)
- Parameter of interest: the relative abundance Q of the peptides

Incomplete labeling

Not all oxygens in the carboxyl terminus are replaced by ^{18}O, due to

- Water impurities – presence of ^{16}O & ^{17}O in the heavy oxygen water

Factors influencing the completeness of labeling

- Speed of atom exchange, quantified by *incorporation rate* λ
- Time τ available for the exchange
- Prevalence of water impurities (π_{16}, π_{17})

Consequences of incomplete labeling

- The isotopic distribution of the labeled peptide shifts by 0, . . ., 4 Da
- It overlaps with the distribution of the unlabeled peptide
- Shift probabilites

$P_0 = P(^{16}O, ^{16}O)$
$P_1 = 2P(^{16}O, ^{17}O)$
$P_2 = 2P(^{16}O, ^{18}O) + P(^{17}O, ^{17}O)$
$P_3 = 2P(^{17}O, ^{18}O)$
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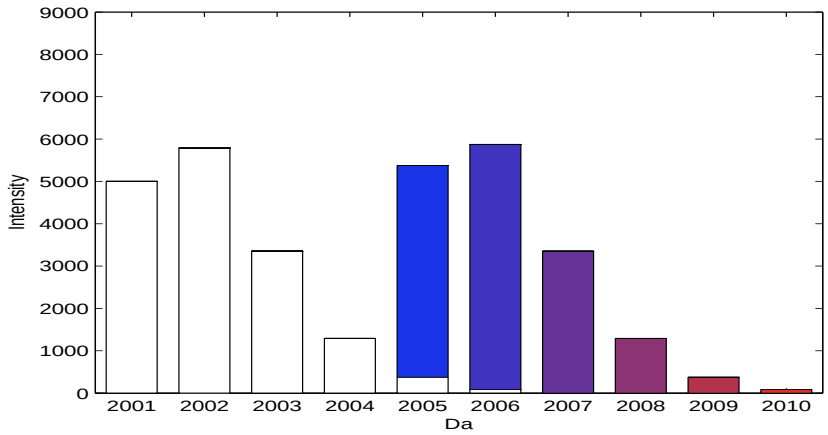

Ignoring the incomplete labeling leads to a biased estimate of Q

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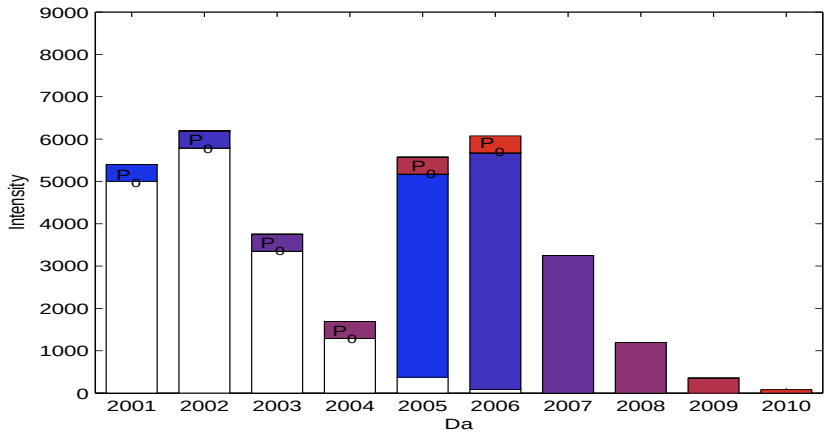

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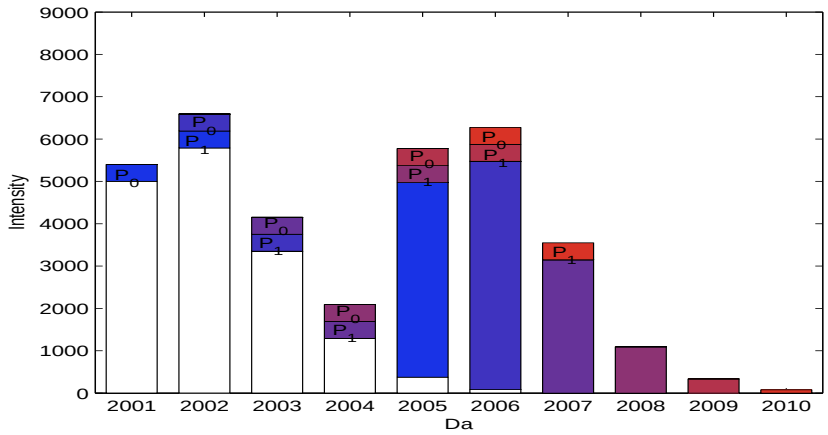

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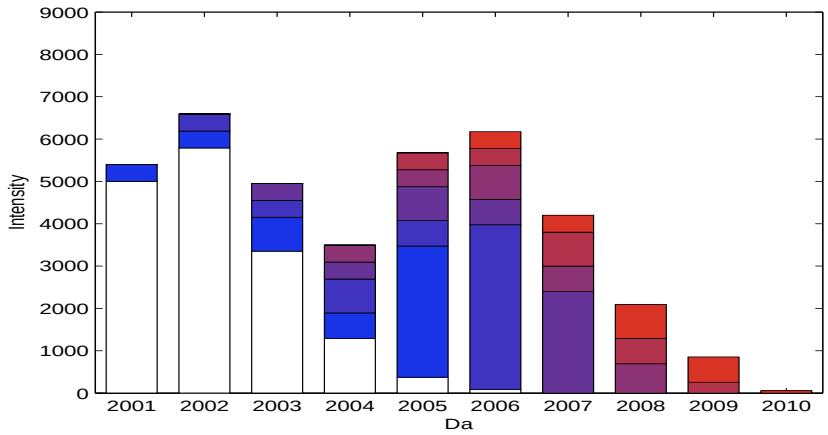

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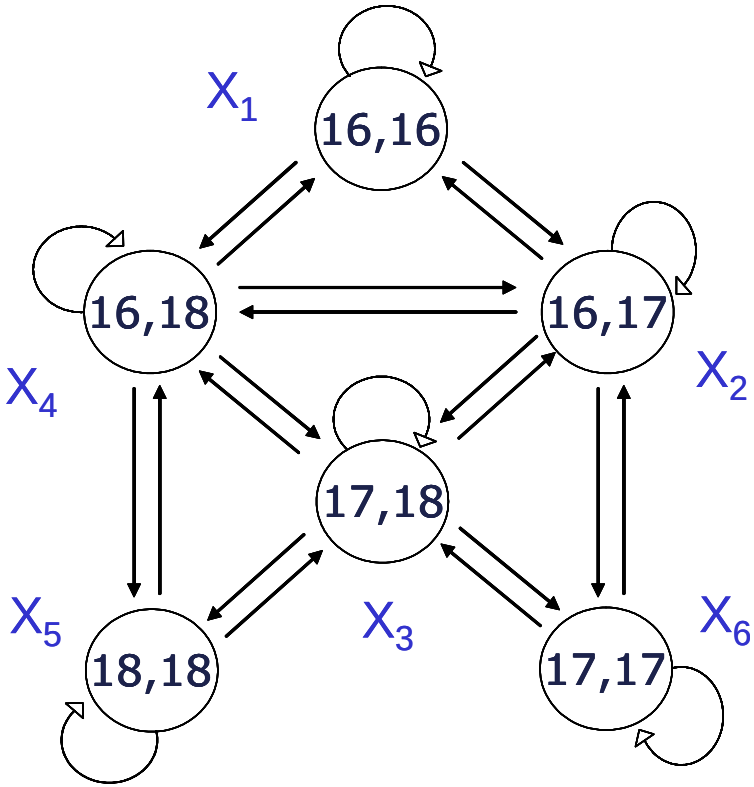
A solution to the incomplete labeling issue

Valkenborg and Burzykowski (2009): a discrete-time Markov-chain model to model shift probabilities P_0-P_4



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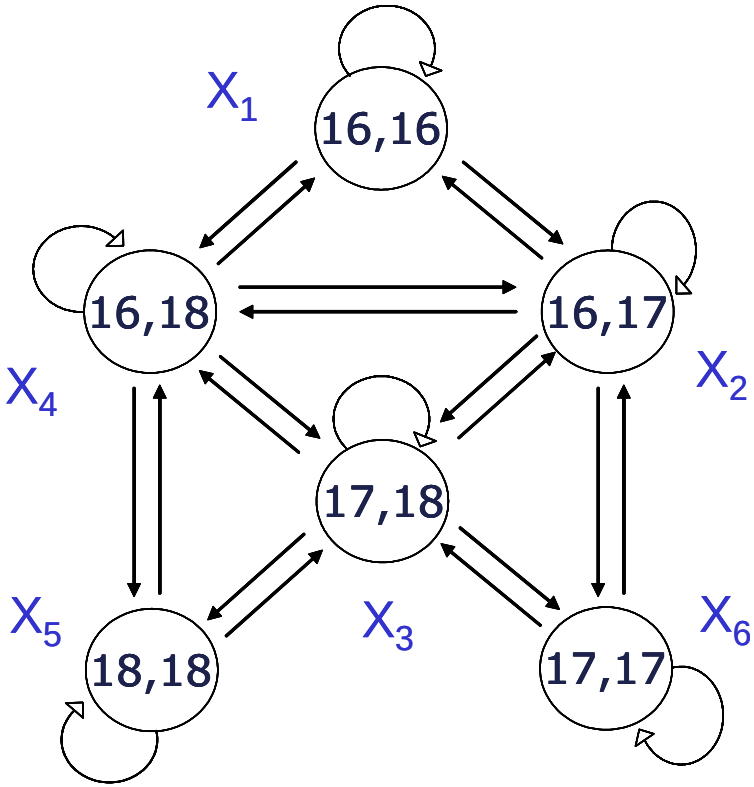


- Transition matrix $\boldsymbol{T}(\pi_{16}, \pi_{17}, \pi_{18})$
- State probability vector

$$\boldsymbol{S}(\tau) = (P(X_1;\tau), P(X_2;\tau), \ldots, P(X_6;\tau))$$

- Initial state $\boldsymbol{S}(0) \equiv \boldsymbol{S}_0 = (1, 0, \ldots, 0)$
- $\boldsymbol{S}(\tau) = \boldsymbol{S}_0 e^{-\lambda\tau} e^{\boldsymbol{T}\lambda\tau}$
- Resulting shift probabilities:

$$P_0(\tau) = P(X_1;\tau),\ P_1(\tau) = P(X_2;\tau)$$
$$P_2(\tau) = P(X_4;\tau) + P(X_6;\tau)$$
$$P_3(\tau) = P(X_3;\tau),\ P_4(\tau) = P(X_5;\tau)$$

Model for the peak intensity

Observed intensity for the jth peak in the ith spectrum (peptide with l isotopic variants):

$$y_{ij} = \mu_{ij} + \varepsilon_{ij},$$

$$\mu_{ij} \equiv \mathrm{E}(y_{ij}) = \begin{cases} H_i R_j + Q H_i \sum_{k=0}^{min(4,j-1)} P_k R_{j-k} & \text{if } 1 \leq j \leq l \\ Q H_i \sum_{k=j-1}^{4} P_k R_{j-k} & \text{if } l+1 \leq j \leq l+4 \end{cases}$$

- Q: relative abundance of the peptide
- H_i: intensity scale for the ith spectrum
- R_j: isotopic ratio
- P_k: shift probability (depends on λ, π_{16}, π_{17}, π_{18}, τ)
- Homoscedasticity: $\varepsilon_{ij} \sim N(0, \sigma^2)$

Proposed extensions

- Heteroscedasticity: $\text{Var}(\varepsilon_{ij}) = \sigma^2 \mu_{ij}^{2\theta}$
- Mixed-effects modeling:

  to capture the technical variability: $H_i \sim N(\mu_H, \sigma_H^2)$

  to capture the biological variability: $Q \sim N(\mu_Q, \sigma_Q^2)$
- Combination (heteroscedastic mixed-effects model)
- Bayesian approach

Bayesian model implementation

- Straightforward implementation of random effects
- Parametrization and prior distributions:
  - non-informative priors for all the parameters
  - Q, H_i, R_j constrained to be positive – logarithmic transformation
  - λ constrained to $(0,20/\tau)$ – Box-Cox transformation
- Practical implementation:
  - WinBUGS through WBDiff: an interface for differentiation equations
  - JAGS: internal matrix exponential function *mexp* in the *msm* module

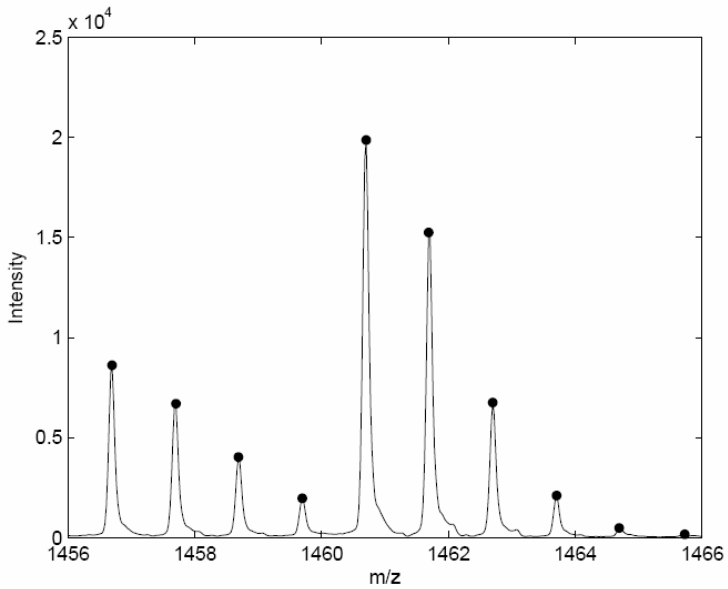
The data

- Mixture of peptides of bovine Cytochrome C (17 protein fragments)
- One part labeled with a stable ^{18}O-isotope, the other unlabeled
- Three unlabeled units mixed with one labeled; the relative abundance of $1/3$...
- ... and *vice versa* – the relative abundance of $3/1$
- Six spectra for each of the relative abundances

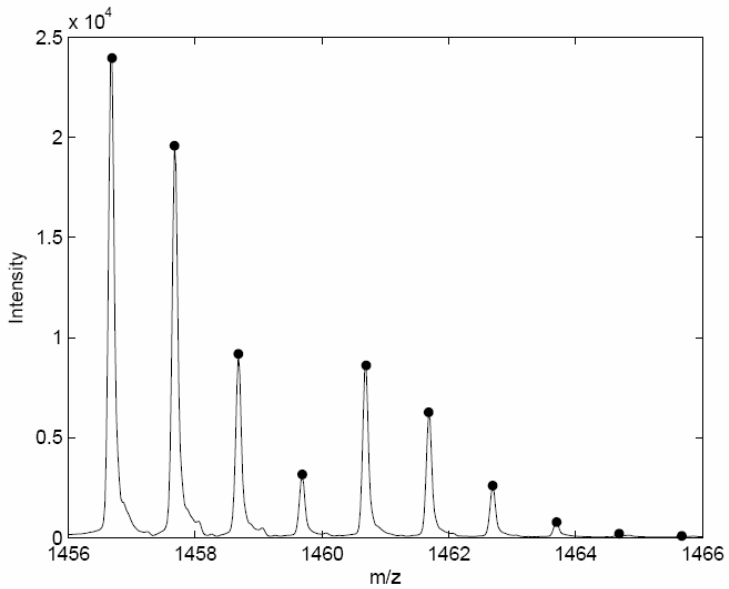
The data

- Peptide with the monoisotopic mass = 1456.66 Da

$Q = 1/3$



$Q = 3/1$



Mean-dependent variance

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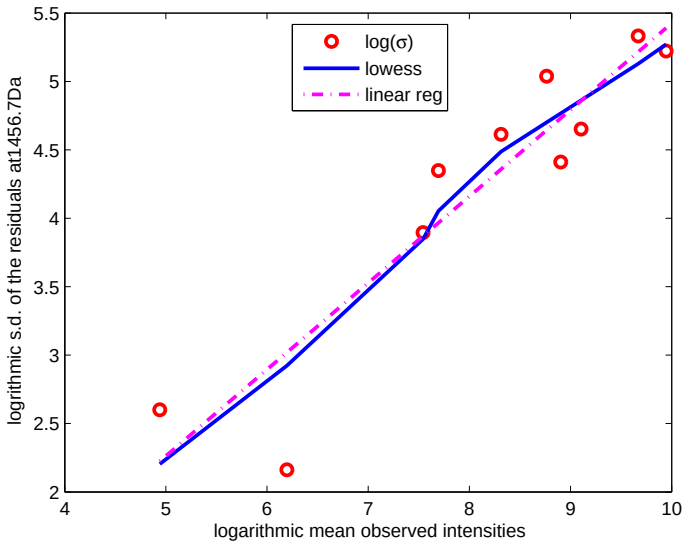

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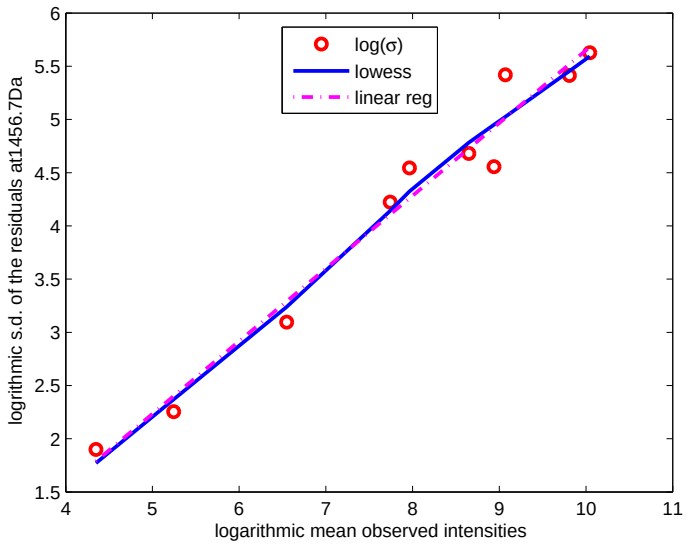

- $0.5 \log\{\mathrm{Var}(\varepsilon_{ij})\} \approx \theta_0 + \theta \log(\mu_{ij}) \rightarrow \mathrm{Var}(\varepsilon_{ij}) \approx \sigma^2 \mu_{ij}^{2\theta}$

Fixed-effects, heteroscedastic model

- Peptide with the monoisotopic mass = 1456.66 Da

Parameter	Ratio 1/3			Ratio 3/1		
	True	Median	95% c.i.	True	Median	95% c.i.
H_1	–	24852.0	(24360.1, 25457.3)	–	8720.3	(8568.3, 8870.5)
H_2	–	22871.3	(22334.2, 23476.7)	–	8819.8	(8670.9, 8973.7)
H_3	–	22667.0	(22179.8, 23214.7)	–	7846.5	(7710.6, 7993.0)
H_4	–	24828.4	(24266.6, 25408.4)	–	10232.0	(10064.8, 10407.4)
H_5	–	19716.8	(19247.8, 20178.4)	–	9812.8	(9645.8, 9989.7)
H_6	–	25179.1	(24635.0, 25806.4)	–	8742.4	(8596.3, 8909.4)
Q	0.33	0.33	(0.32, 0.34)	(2.4)	2.4	(2.3, 2.4)
$\lambda\tau$	–	8.8	(5.5, 11.2)	–	11.5	(10.3, 13.2)
σ	–	0.21	(0.10, 0.51)	–	0.43	(0.17, 1.23)
θ	–	0.77	(0.66, 0.86)	–	0.66	(0.53, 0.77)
R_1	0.7933	0.7770	(0.7611, 0.7926)	0.7933	0.7763	(0.7651, 0.7868)
R_2	0.3567	0.3416	(0.3335, 0.3490)	0.3567	0.3430	(0.3368, 0.3493)
R_3	0.1166	0.1030	(0.1002, 0.1059)	0.1166	0.1015	(0.0987, 0.1045)
R_4	0.0306	0.0275	(0.0261, 0.0292)	0.0306	0.0245	(0.0235, 0.0256)
R_5	0.0068	0.01115	(0.0104, 0.0119)	0.0068	0.0070	(0.0066, 0.0076)

Homoscedastic mixed-effects model (random H & Q)

- Peptide with the monoisotopic mass = 1456.66 Da

Parameter	Ratio 1/3			Ratio 3/1		
	True	Median	95% c.i.	True	Median	95% c.i.
μ_H	–	22978.2	(20713.2, 24835.0)	–	8962.2	(8225.2, 9617.3)
σ^2_H	–	4538005.5	(1515783.0, 21798794.0)	–	884115.12	(302543.88, 3915923.5)
μ_Q	0.33	0.33	(0.27, 0.44)	(2.4)	2.4	(2.3, 2.4)
σ^2_Q	–	0.0052	(0.0017, 0.0254)	–	0.0056	(0.0019, 0.0245)
$\lambda\tau$	–	7.4	(6.8, 8.0)	–	10.4	(9.4, 11.8)
σ	–	158.6	(131.0, 199.1)	–	128.7	(106.0, 160.3)
R_1	0.7933	0.7905	(0.7831, 0.7977)	0.7933	0.7761	(0.7695, 0.7826)
R_2	0.3567	0.3494	(0.3436, 0.3555)	0.3567	0.3396	(0.3341, 0.3448)
R_3	0.1166	0.1062	(0.1004, 0.1114)	0.1166	0.1004	(0.0950, 0.1052)
R_4	0.0306	0.0392	(0.0313, 0.0535)	0.0306	0.0246	(0.0198, 0.0295)
R_5	0.0068	0.0090	(0.0040, 0.0188)	0.0068	0.0062	(0.0040, 0.0114)

Concluding Remarks

- Frequentist estimation works well (results not shown)
- Bayesian approach is feasible
- Nest step: heteroscedasticity AND random effects

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