Statistical approach to absolute protein quantification

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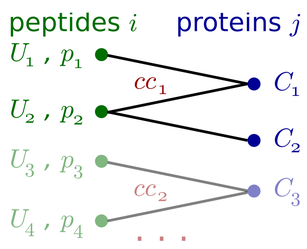
Abstract

We propose a statistical approach to label-free protein quantification with three main advantages compared to existing methods. (i) Peptide intensities are modeled as random quantities, allowing to account for the uncertainty of these measurements. (ii) Our Markovian-type model for bipartite graphs ensures transparent propagation of the uncertainties and reproducible results. (iii) The problem of peptides mapping to several protein sequences (often neglected in other models) is addressed automatically according to our statistical model. The performance of our model is shown on three control datasets and compared to the results of two common approaches for protein quantification: APEX [1] and "top3" [2].

Model

Notation



U_i : intensity score *(given)*
p_i : identification score *(given)*
C_j : concentration *(unknown)*
$\Rightarrow$ latent variable
cc_r : connected component with
- n_r peptides
- m_r proteins

Furthermore we use two "distance" measures

- d_i is the number of proteins having a common edge with peptide i
- d_{ik} is the number of proteins having a common edge with peptide i and peptide k

and define $\underline{U}_r$ as the vector of intensities of all peptides in the connected component r; $\mathfrak{Sigma}_{\underline{U}_r} = \mathrm{Cov}(\underline{U}_r)$ and $\underline{\alpha} = \alpha(1, \ldots, 1)^\intercal$.

Markovian-Type Assumption

Peptides belonging to the same connected component are independent given their matching proteins. This implies that dependencies among peptides are exclusively due to their common proteins. Furthermore, we make a Markovian assumption (for graphical models) which states that only the neighboring proteins matter in the conditional distribution for the peptides (see also [3]).

Model

$C_1, C_2, \ldots, C_{m_r}$ are i.i.d. with $\mathbf{E}[C_j] = 0$ and $\mathrm{Var}(C_j) = 1$.

We propose the following model for the peptide intensities:

$$U_i = \alpha + p_i \beta \, d_i^{-\frac{1}{2}} \sum_{j \in Ne(i)} C_j + \epsilon_i$$

where $\epsilon_1, \epsilon_2, \ldots, \epsilon_{n_r}$ are i.i.d. with $\mathbf{E}[\epsilon_i] = 0$ and $\mathrm{Var}(\epsilon_i) = \tau^2$. The elements of the covariance matrix of U are then given by

$$\mathrm{Cov}(U_i, U_k) = \begin{cases} p_i \, p_k \, \beta^2 \frac{d_{ik}}{\sqrt{d_i}\sqrt{d_k}} & i \neq k \\ p_i^2 \, \beta^2 + \tau^2 & i = k \end{cases}$$

and the covariance between C_j and U_i is

$$\mathrm{Cov}(C_j, U_i) = \begin{cases} 0 & \text{if there is no edge between } i \text{ and } j \\ p_i \, \beta \frac{1}{\sqrt{d_i}} & \text{if there is an edge between } i \text{ and } j \end{cases}$$

Predicting the protein concentrations

Assume we are working on the first connected component, then the corresponding protein concentrations are given by

$$\mathbf{E}[C_j | \underline{U}_1] = (\underline{U}_1 - \underline{\alpha})^\intercal \, \mathfrak{Sigma}_{\underline{U}_1}^{-1} \begin{pmatrix} \mathrm{Cov}(C_j, U_1) \\ \mathrm{Cov}(C_j, U_2) \\ \vdots \\ \mathrm{Cov}(C_j, U_{n_1}) \end{pmatrix}$$

Parameter estimation

Maximum likelihood estimation (MLE)

$$\underline{U}_r \sim \mathcal{N}_{n_r}(\underline{\alpha}, \mathfrak{Sigma}_{\underline{U}_r})$$
$$f(\underline{U}_r; \alpha, \beta, \tau^2) = |2\pi \mathfrak{Sigma}_{\underline{U}_r}|^{-1/2} \exp\left(-\frac{1}{2}(\underline{U}_r - \underline{\alpha})^\intercal \, \mathfrak{Sigma}_{\underline{U}_r}^{-1} (\underline{U}_r - \underline{\alpha})\right)$$

The negative log-likelihood can then be written as

$$-\sum_{r=1}^{R} \log\left(f(\underline{U}_r; \alpha, \beta, \tau^2)\right)$$

which has to be minimized w.r.t. $\alpha, \beta, \tau^2 > 0$.

Least squares approach (LSA)

Estimate α (mean) and the covariance matrix ($\widehat{\mathfrak{Sigma}}_{\underline{U}_r}$) from the data. Use the off-diagonal elements of $\widehat{\mathfrak{Sigma}}_{\underline{U}_r}$ to estimate β:

$$\sum_{r=1}^{R} \sum_{\substack{i \neq k \\ i,k \in cc_r}} \left(\left(\widehat{\mathfrak{Sigma}}_{\underline{U}_r}\right)_{ik} - p_i \, p_k \, \beta^2 \frac{d_{ik}}{\sqrt{d_i}\sqrt{d_k}} \beta^2 \right)^2 \stackrel{!}{=} \underset{\text{w.r.t. } \beta^2}{\text{minimize}}$$

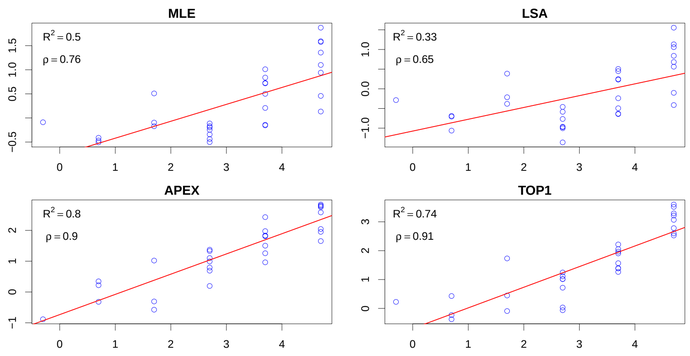
With the diagonal elements and $\hat{\beta}$ one can compute the estimate for τ^2:

$$\sum_{r=1}^{R} \sum_{i=1}^{n_r} \left(\left(\widehat{\mathfrak{Sigma}}_{\underline{U}_r}\right)_{ii} - p_i^2 \, \hat{\beta}^2 - \tau^2 \right)^2 \stackrel{!}{=} \underset{\text{w.r.t. } \tau^2}{\text{minimize}}$$

Results

UPS2 proteomic standard [4]

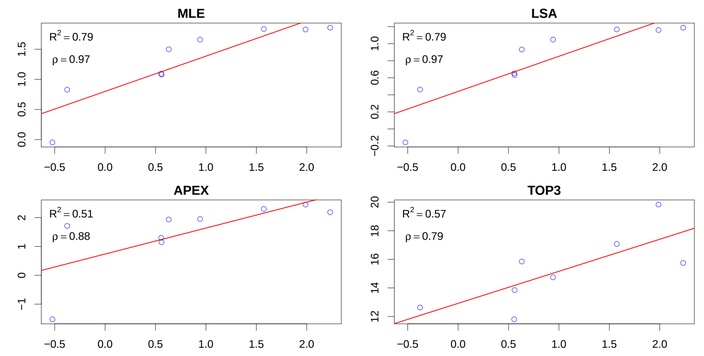
Shotgun (Orbitrap) experiments on several injections of the (pure) UPS2 mixture (Sigma), containing 48 proteins in 6 known concentrations. We combine the results from all replicates to compare the performance of the quantification methods.



known concentrations versus computed scores

9 spiked proteins [5]

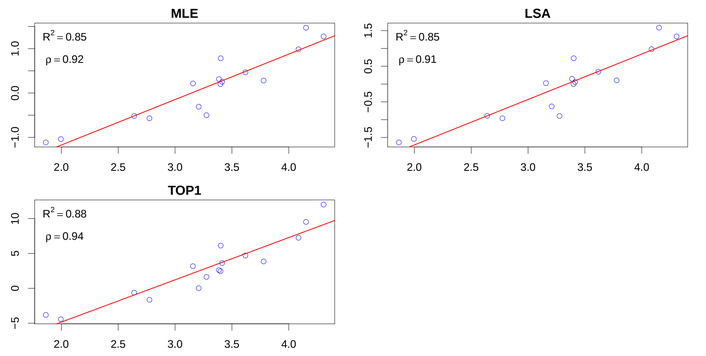
Shotgun (Orbitrap) experiments on 9 non-human proteins spiked into human (K562) cell lysate in 3 different concentrations and analyzed in 6 injections each. We compare the performance of the different methods in one of the mixtures (all technical replicates combined).



known concentrations versus computed scores

Leptospira interrogans [6]

Selected reaction monitoring (SRM) experiment on 16 *L. interrogans* proteins under 3 conditions (with 3 technical replicates each). The proteins were experimentally quantified using AQUA peptides [7]. We compare the performance of the different methods for one of the conditions (all technical replicates combined).



known concentrations versus computed scores

Outlook & Implementation

Conclusions

The results from our model are competitive with other widely used approaches for protein quantification.

The main advantage of using the least squares approach would be to save computation time. However, the results so far show that the MLE approach often leads to better predictions. It might still be interesting to use the LSA to find good starting values for the MLE.

Our model is not designed to work with a particular setting/machinery, but can handle different types of intensity measures for the peptides.

Outlook

The presented approach does not outperform existing tools in their performance. However, it potentially holds two advantages:

- Our model deals *per se* with shared peptides (instead of discarding them) and might thus bring further insight for organisms with an important amount of shared peptides.
- Our method does not rely on spectral counts nor one the proportion of seen versus unseen peptides, and can thus also be used in directed MS experiments or targeted proteomics.

R Code

Our method has been implemented in R with the following packages/program versions:

- R version 2.13.0 (2011-04-13), `x86_64-unknown-linux-gnu`
- Base packages: base, datasets, graphics, grDevices, methods, stats, utils
- Other packages: sfsmisc 1.0-14, xtable 1.5-6

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