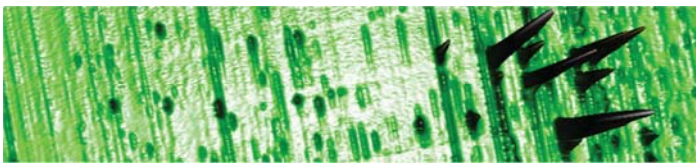
Biophysical Rationale and Quantitative Benefits of using Linear Mixed Effect Models to summarize Transitions of Peptides to Protein abundances in SRM

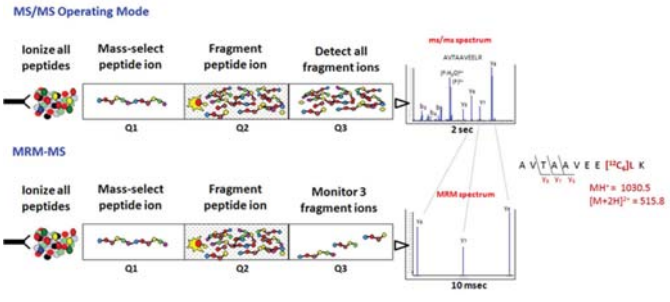


What is SRM / Field of application

- SRM
  - stands for Selective Reaction Monitoring.
  - is a mass spectrometry based method for quantitative measurement of target proteins.
- MRM™ is a trademark of AB SCIEX, but has the same principle.
- Fields of application in pharmaceutical research:
  - Pharmacodynamic: quantitation of proteins if the there is no antibody available, but target protein quite abundant (e.g. study of apoliproteins from rabbit plasma in dyslipidemic models).
  - Pharmacokinetic of therapeutic peptides.

Content

How does it work?



by Broad Institute, MA

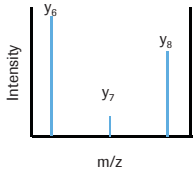
PROBLEM IN CURRENT APPLICATIONS

Biophysical Rationale

How are protein abundances determined now and what is the problem?

- It is still common to relate the relative abundance of a protein by taking the
  - Sum
  - Mean, or
  - Median

  of the peak Areas of transitions.
- Problem arises if 1-2 transition are not determined because it is
  - An outlier (e.g. by contamination of signal)
  - Below limit of Quantitation.



Transitions-Peptide-Protein Model I
Assumptions

- After trypsination, a peptide's concentration should be function of the originating proteins concentration:

  $c(\text{Peptide}) = c(\text{Protein}) * f_{\text{Tryps, p}} * \varepsilon_1 \qquad f_{\text{Tryps, p:}} \in [0,1]$
- The number of ionized peptides after Electron Spray Ionization is a function of the peptides concentration:

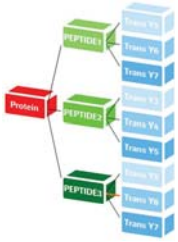
  $n(\text{Peptide}) = c(\text{Peptide}) * f_{\text{Ion, p}} * \varepsilon_2$
- The AUC of transitions over MS/MS and fragmentation is a function of the number of ES – ionized peptides passing the mass selection in MS and the fragmentation factor f_{frag}.

  $A(\text{Transition}) = n(\text{Peptide}) * f_{\text{frag, p}} * \varepsilon_3$
- Combining the three terms forms a relation between A and protein abundance:

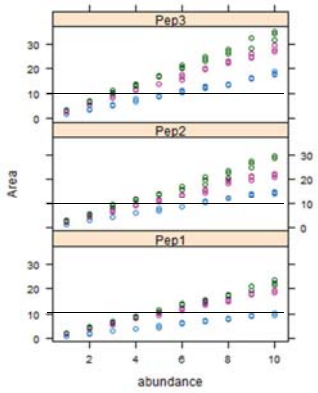
  $A(\text{Transition}) = c(\text{Protein}) * f_{\text{Tryps, p}} * f_{\text{Ion, p}} * f_{\text{frag}} \varepsilon_*$

Transitions-Peptide-Protein Model II
Assumptions

- After log-transformation, factors become addends:
  $\log(A(\text{Transition})) = \log(c(\text{Protein})) + \log(f_{Tryps,\,p} * f_{Ion,\,p}) + \log(f_{frag}) + \log(\varepsilon_*)$
  $\log(A(\text{Transition})) = \text{RelAbundanceProtein} + \text{PeptideEffect} + \text{FragEffect} + \varepsilon^*$
- Assumptions:
  - PeptideEffect ~ $N(0, \sigma_p)$
  - FragEffect ~ $N(0, \sigma_{f(p)})$
  - $\varepsilon \sim N(0, \sigma_e)$
- Example of a hierarchical, mixed effect model where a transition is nested in a peptide which is nested in protein.
- log(A(Transition)) ~ 0 + SampleID + (1| PeptideID/TransitionID)



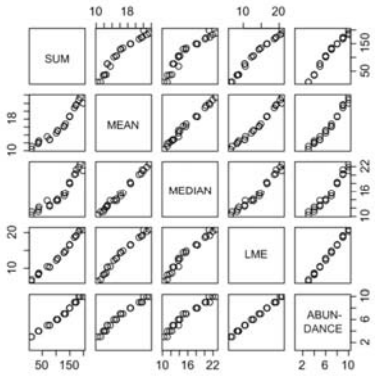
Data Simulation: Study Design



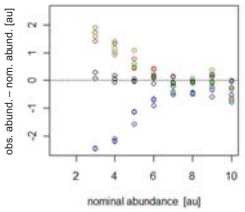
- **Data Simulation:**
  - True, nominal abundance from 1 to 10 au; protein with 3 peptides (boxes), each with 3 transitions (colors).
  - Transition Areas are computed from imaginary ionization and fragmentation factors multiplied with nominal abundance.
  - 'Below Limit of Quantitation' is simulated by setting all areas below 10 to NA.

QUANTITATIVE BENEFIT-DATA SIMULATION

Data Simulation: Mutual Relations



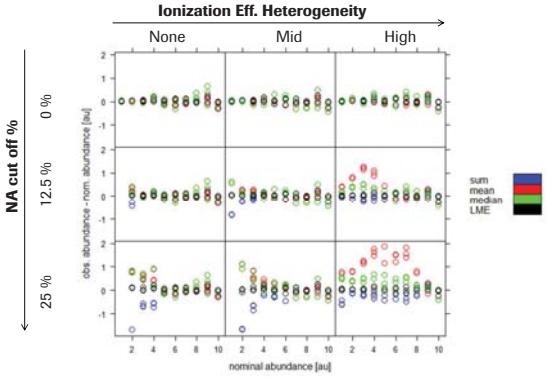
Data Simulation: Bias Plot



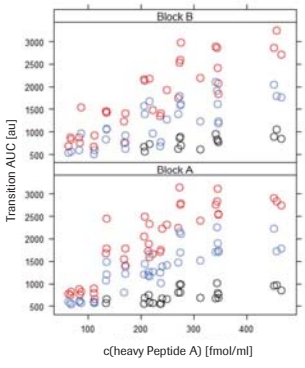
- Bias Plot (observed-nominal) ~ nominal
- At low nominal abundances (where most NA occur,)
  - Sum of transitions deviates strongly from 0 residual (> 100 % off in relative terms), is biased towards reporting lower observed abundances than true nominal abundance
  - mean, or median of transitions ALSO deviate from 0 with a positive bias.
  - **LME** (black) shows no bias and has smallest residual.

QUANTITATIVE BENEFIT – REAL DATA

Influence of NA cut off % and Ionization Efficiency Heterogeneity on Bias

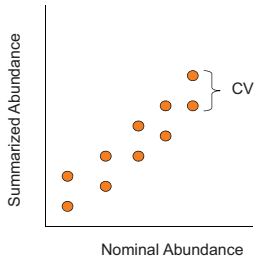


Real Data: Study Design



- Approach works best in poor data
- Spike-in of defined concentrations of "heavy-labeled" peptide A in human plasma. No interference with endogenous "light" peptide A.
- Each sample measured with three transitions (**o**, **o**, **o**) in two blocks (A & B).
- Transition **o** has low ionization efficiency and is not available in all samples.

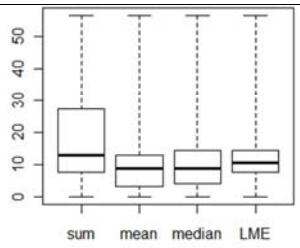
Example Batch to Batch repeatability



Real Data: Quantitative Characteristics from Spike-in

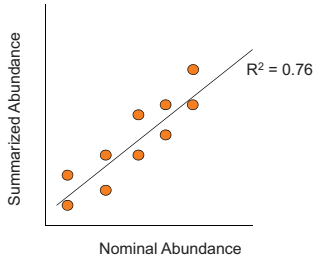
Accuracy: R^2 0.71 0.69 0.65 0.73*

Repeatability: CV



* R^2 increased to 0.77 with "Block" as random factor.

Example Accuracy: R^2



Summary & Conclusions I
How to infer protein abundance from SRM transitions

- SRM for quantitation of proteins and peptides:
  - Very useful if the there is no antibody available, but target protein quite abundant.
- Biophysical Rationale:
  - Hierarchical, mixed effect model explains best that a transition is nested in a peptide which is nested in protein.
- Data Simulation: At low nominal abundances (where most NA occur)
  - Sum of transitions deviates strongly from 0 residual, is biased towards reporting lower observed abundances than true nominal abundance.
  - mean, or median of transitions ALSO deviate from 0 report a positive bias
  - **LME** (black) shows no bias and has smallest residual.
  - The higher the percentage of NA, the more important it is to use LME.

Summary & Conclusions II

- Real Data Example:
  - Accuracy: LME is better than sum, mean or median, especially if experimental conditions (e.g. blocking factors) are considered.
  - Repeatability: LME provides more repeatability compared to sum of transitions, but is not better than mean or median

The Contributors

- Anton Belousov
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- Heinz Döbeli
- Arno Friedlein
- Jens Lamerz

... And beyond.

Protein Significance Analysis in Selected Reaction Monitoring (SRM) Measurements*

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- Using LME has more advantages:
  - In a variety of experimental designs, LMEM combined with SRM have increased power compared to naïve t-tests.
  - Extensions can be made to specify heterogeneity of biological and technical variance components across features or conditions.

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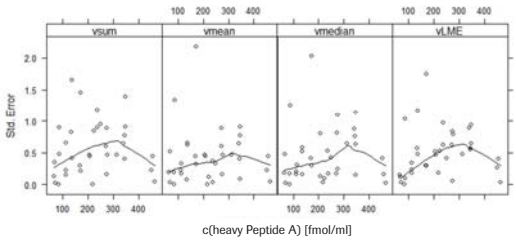
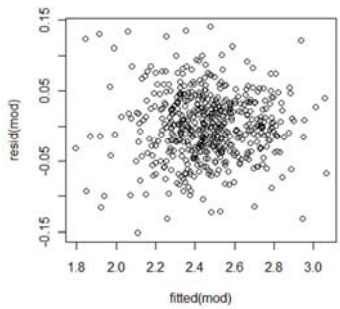
Standard Error vs. Spiked in Concentration



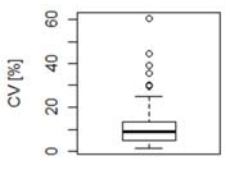
Model Diagnostics
Good characteristics of unexplained variance



Why?

Biological & Technical Feasibility
Free Peptide A in Plasma

- Result II: Repeatability of technical replicates is very good (median CV 9 %)



	CV [%]
Min	1.2
Q1	5.1
Median	8.9
Q3	13.4
Max	60.8

Acknowledgements

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RESEARCH PAPER

Mass Spectrometry Based Quantification of CYP Enzymes to Establish *In Vitro-In Vivo* Scaling Factors for Intestinal and Hepatic Metabolism in Beagle Dog

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