Computational and Statistical Issues in the Analysis of Metabolic Profiling Data

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What is Metabolomic Profiling: A Statistican’s View?

- Measure many analytes from each biological sample
- Need to measure analytes over a very wide range of concentrations in the same sample
- Metabolic profile = estimated concentrations of many analytes
- What parts of the profile are important?

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Metrology

- Comparing two large values of an analyte, one from each sample, we might use the ratio x/y.
- Log(x/y) = log(x) – log(y)
- The log ratio is often better behaved statistically.

- When one or both of x and y is small, the ratio no longer makes sense.
- An increase from 1000 to 1200 is a 20% increase, or a ratio of 1.2
- An increase of 0 to 1000 has no percentage increase or ratio.
- Which is biologically more important?
- Log(0) is not defined.

The Glog Transform

- We use a transformation that looks like the log at high levels, but is defined and well behaved at low levels, even 0 or negative.
- This often helps regularize data.
- $\mathrm{Log}((y-\alpha)+\sqrt{[(y-\alpha)^2+\lambda]})$
- Defined for all y, monotonic, linear at 0, log for high levels

Experimental Design

- No matter how much is measured from one biological sample, the size of the experiment is the number of distinct samples/organisms.
- If you need 100 people in each group to test for one response, you need 100 to test for many.

Technologies

- Mass spectrometry of many varieties
- Many possible separation technologies with many types of detectors
- NMR spectroscopy

Statistics and Bioinformatics

- Data handling and processing of spectra, including baseline estimation
- Transformation to appropriate scales
- Identification of specific compounds or spectral regions that discriminate

- Identification of compounds from spectra
  - Multiple peaks per compound (NMR)
  - Multiple compounds per mass in MS
- Identification by computation and by database searches

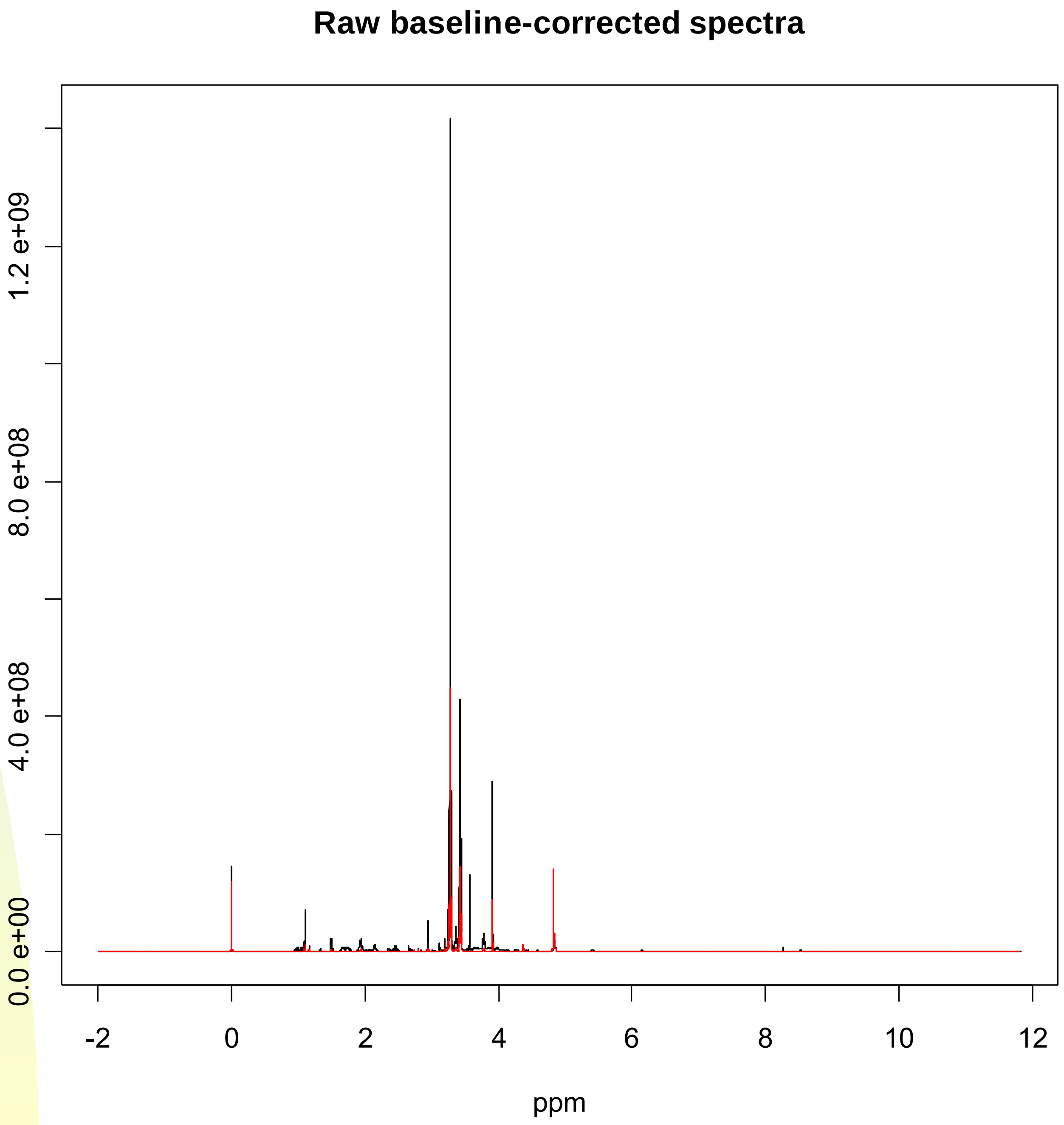
Multivariate Methods

- Methods such as PLS can identify complex biomarkers.
- Investigate other methods of dimension reduction and classification
  - PCA, PLS, SOM, stepwise selection
  - LDA, QDA, PLS, NN, Logistic Regression

- Critical to get the statistical model and initial data processing right.
- Many methodologies assume stability of variance
- Those that don’t assume it are often more effective when this stability exists
- Carefully chosen data transformations can accomplish this

NMR Spectroscopy for Metabolomic Profiling

- Many computational and statistical challenges.
  - Baseline correction
  - Peak shifting
  - Multiple peaks per compound
- We are currently exploring methods of analysis for this tool.



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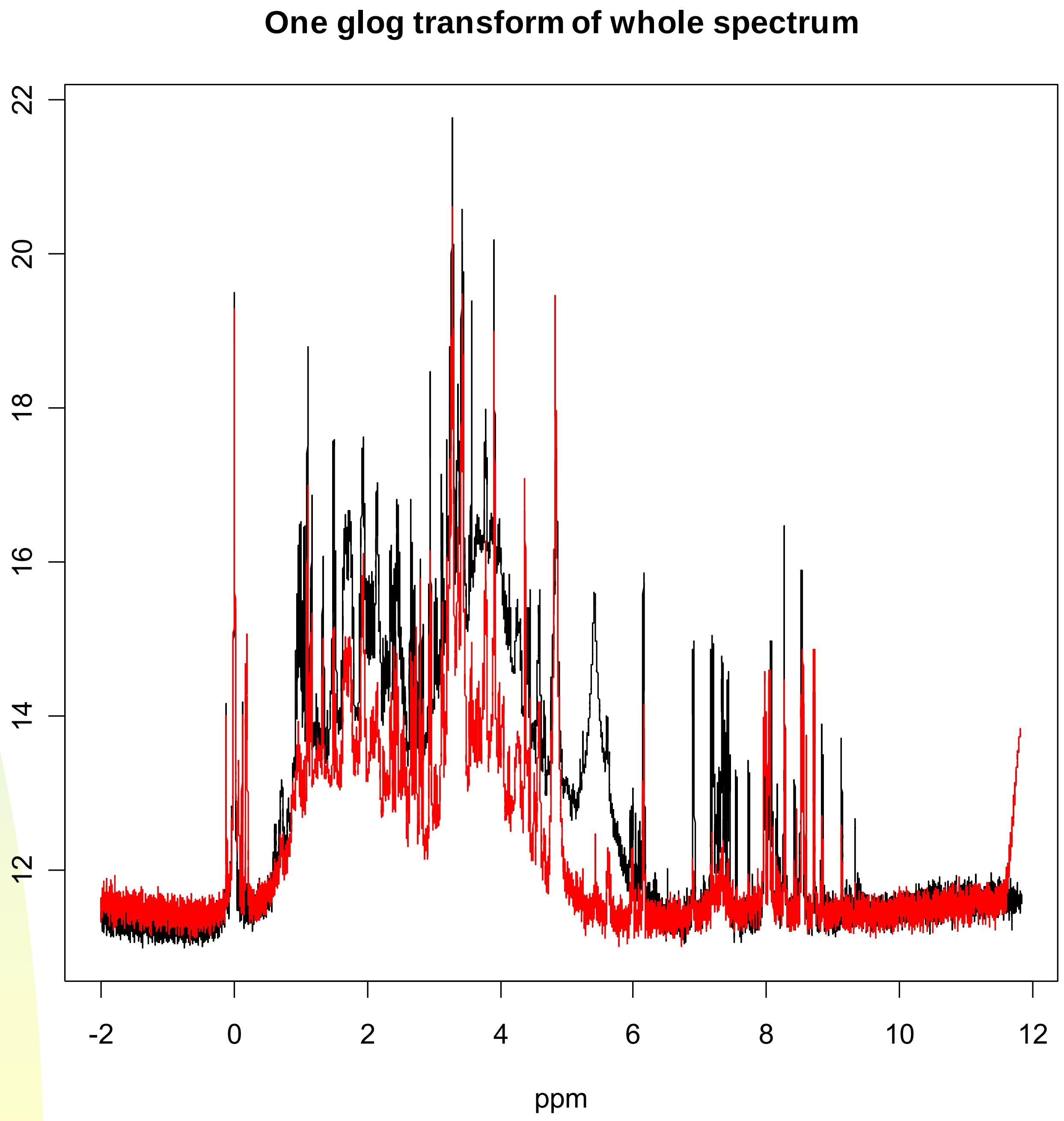

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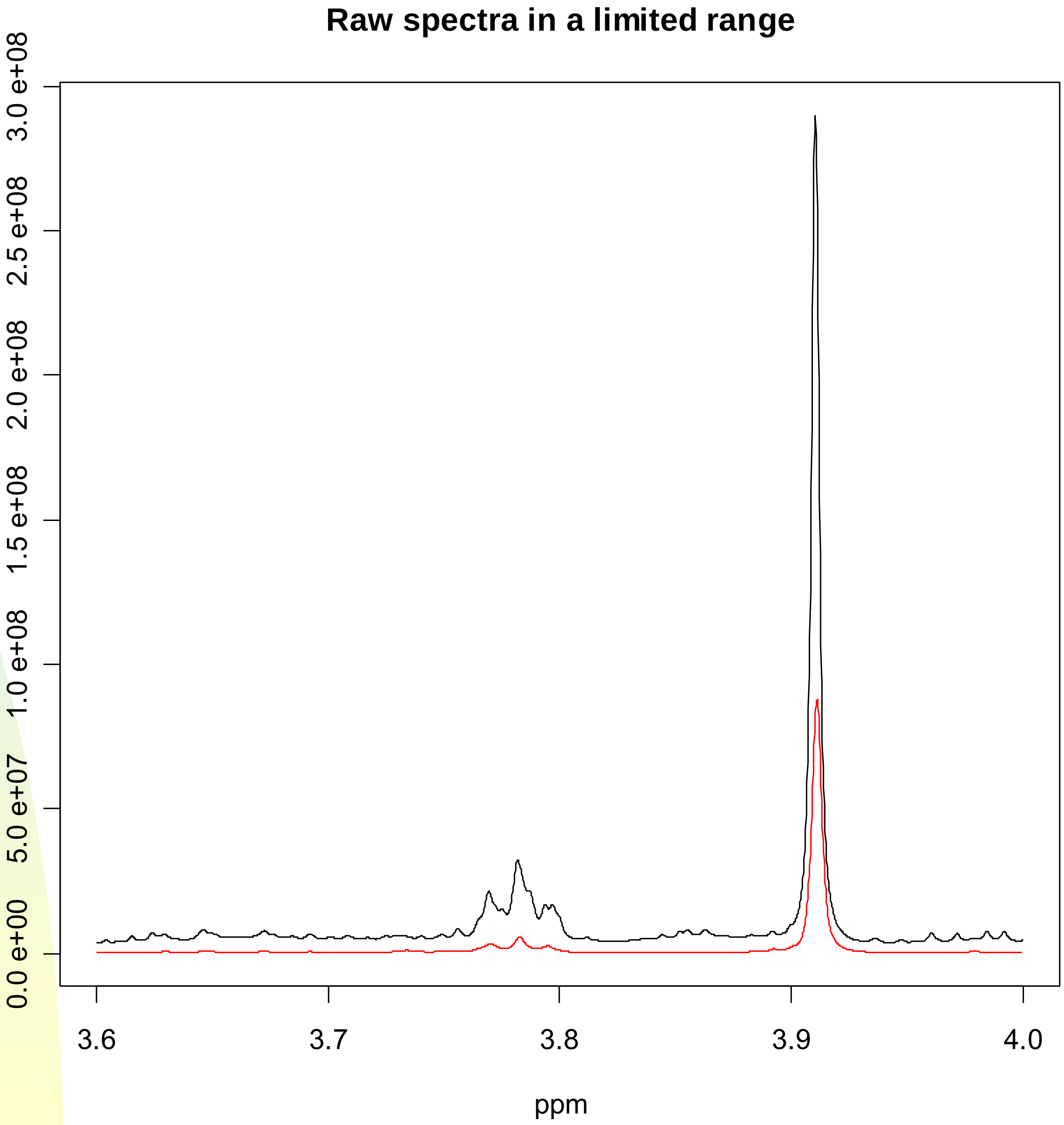

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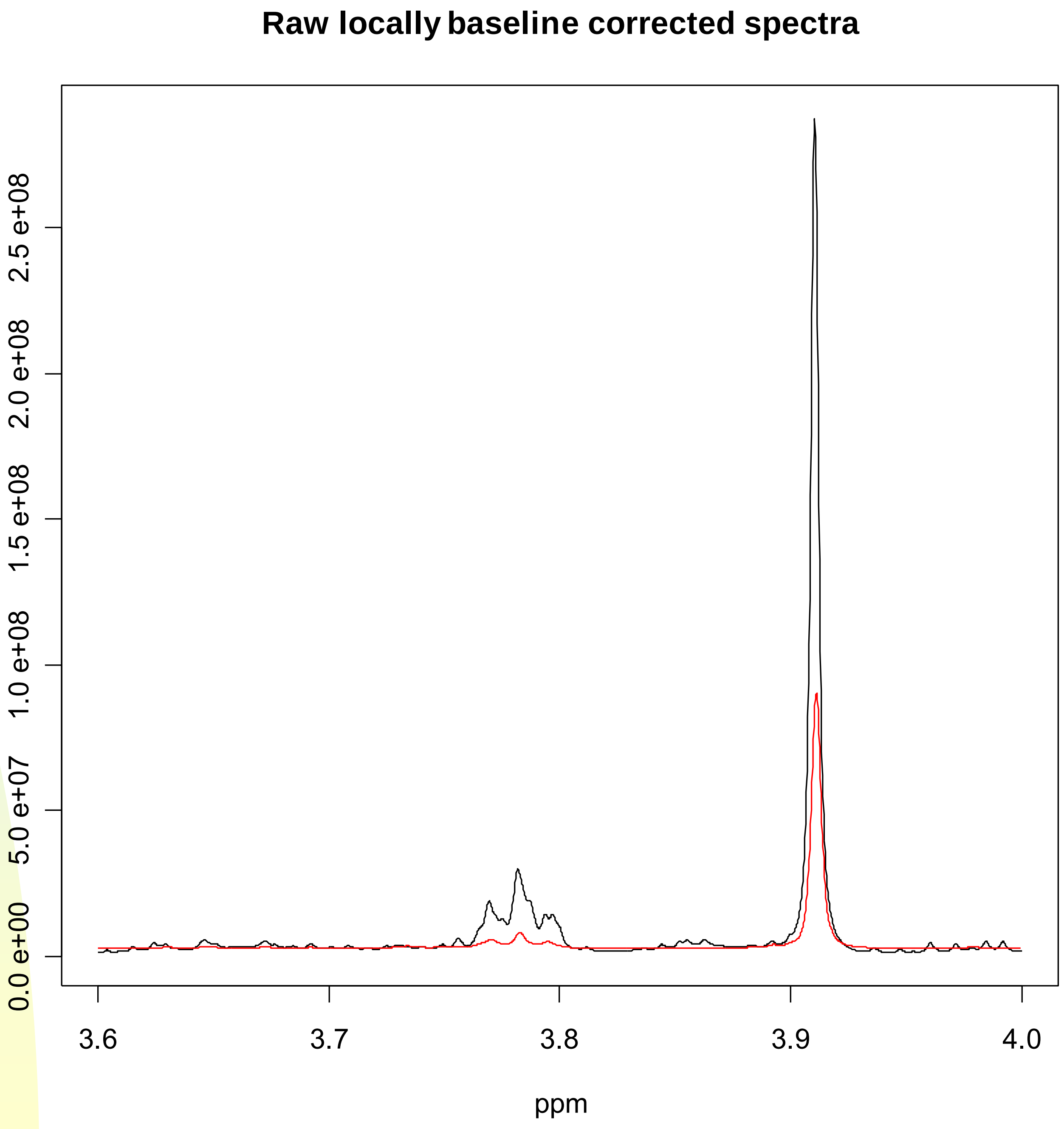

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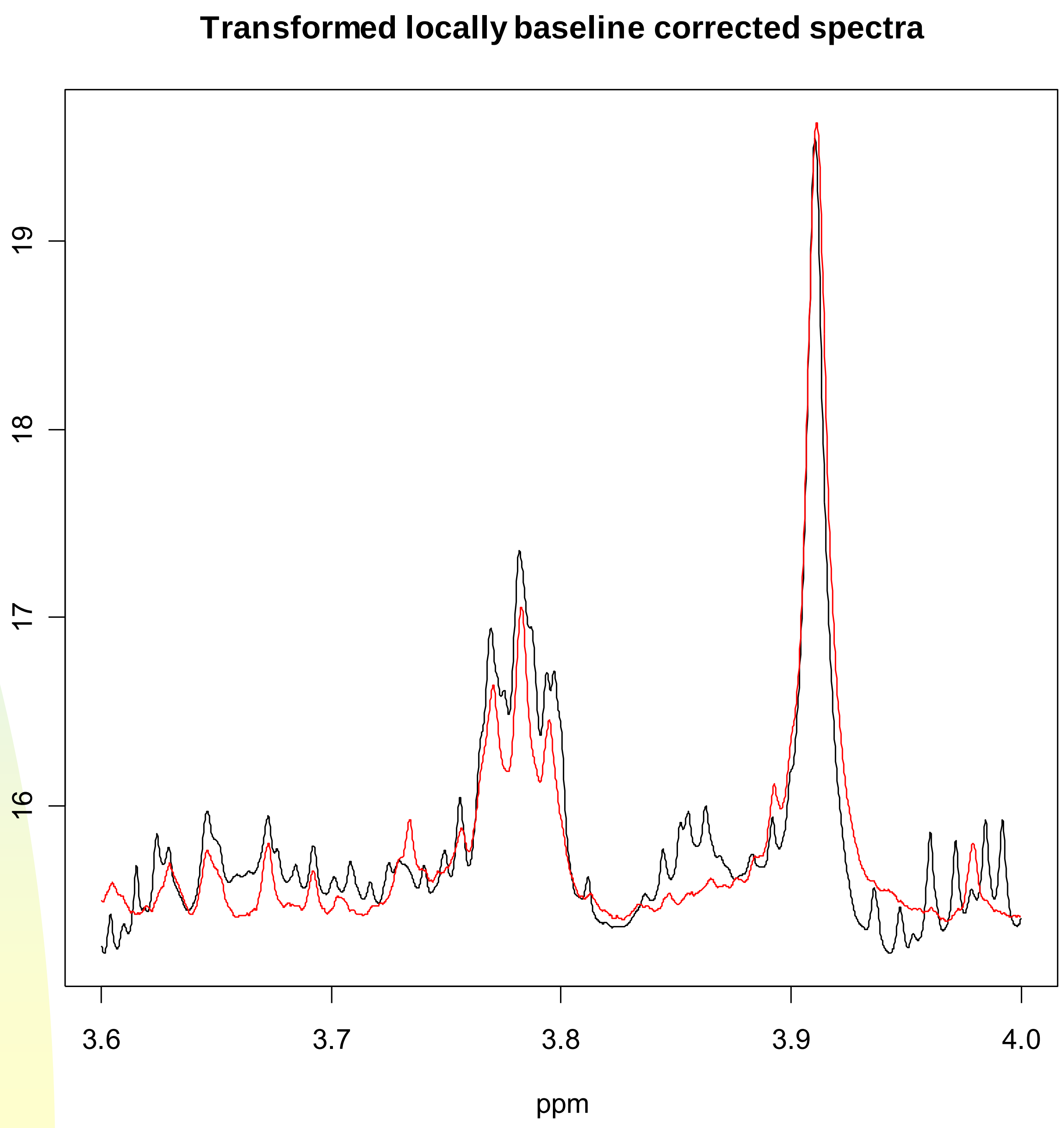

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Tentative Conclusions about NMR Spectroscopy

- The baseline needs to be estimated in an adaptive but statistically principled fashion.
- The data need to be transformed to approximate stability.
- Normalization after transformation is likely to be necessary.
- We need to use this powerful tool to identify biomarkers not easily identifiable otherwise.

Conclusions

- Data handling and statistical design and analysis are both important enabling technologies.
- Similar issues will be apparent in any spectroscopic technology.
- Collaborations between many disciplines will be needed to advance metabolic profiling.