Overview of the NC State Approach to Genomic Dose-Response Modeling

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Overview/preview of statistical procedures

Quality Control

- For sequence-based transcriptomic technologies, threshold individual genes based on expression level
- Outlier checks
- Compare control samples to all other control samples

Normalization

- Currently done per-experiment, e.g. using DESeq2 for sequence-based transcriptomics

Overview/preview of statistical procedures

Testing

- For statistical flags, we use simple rank-based procedures (see later)
- For differential expression analysis, we use shrinkage-based methods (for example, DESeq2, limma)

Multiple testing

- False discovery control

Dose-response curve fitting

- Highly reliant on 4-parameter (Hill) logistic fitting, or 3-parameter if that makes more sense in the context. With more data, gain-loss modeling

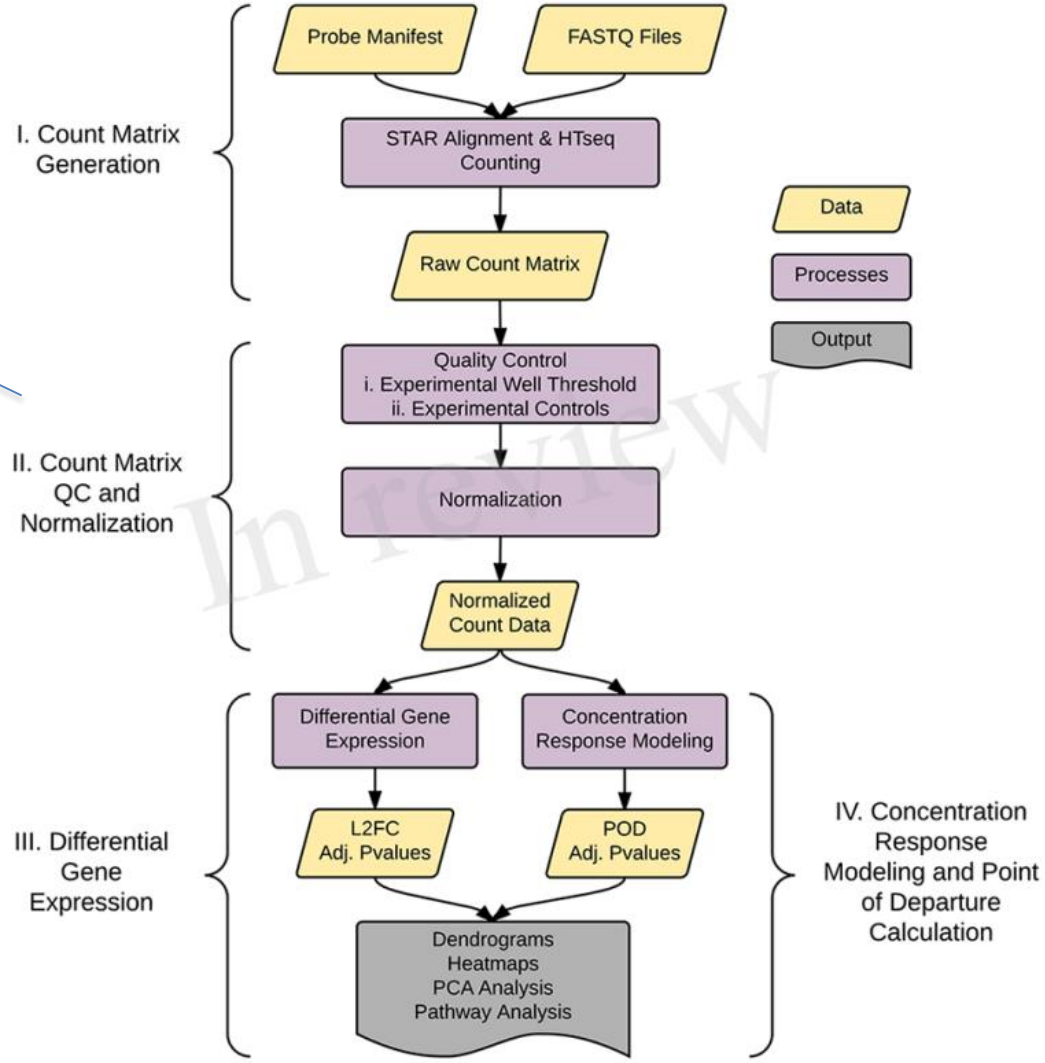
A series of choices and tests

- When dealing with gene expression dose-response data, natural tension among *statistical flags*, *testing,* and *modeling*
- Some of the pipeline reflects a specific sequencing technology
- Potential concern that controls may differ from dosed conditions for technical reasons

Pipeline overview

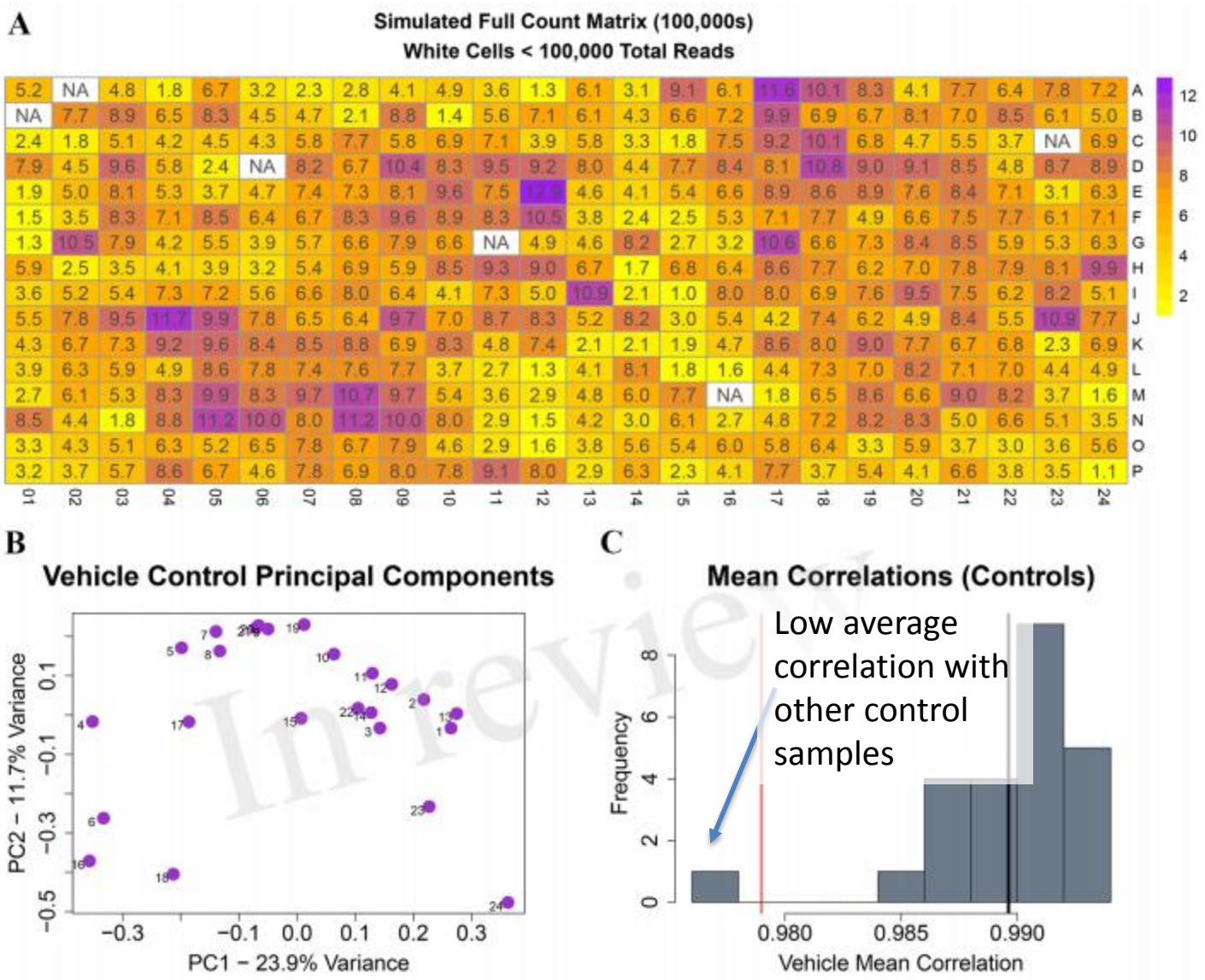
https://github.com/jshousephd/HT-CBA

Somewhat platform-specific

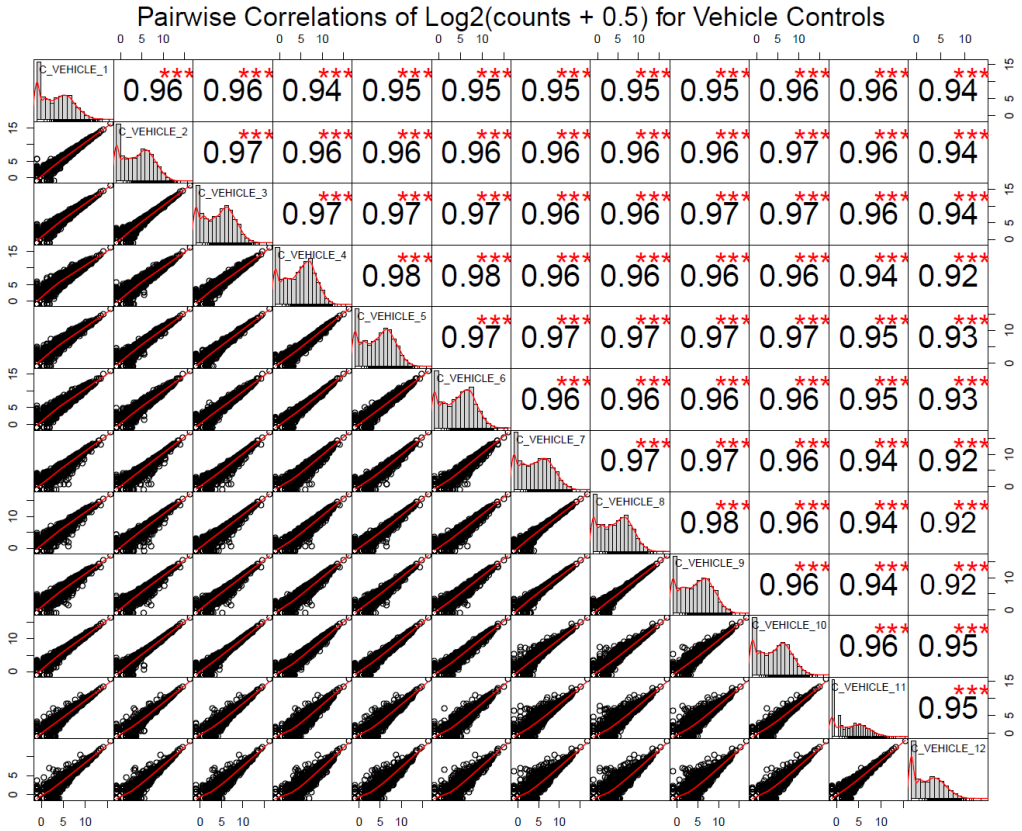


Pre-analysis Quality Control of Samples

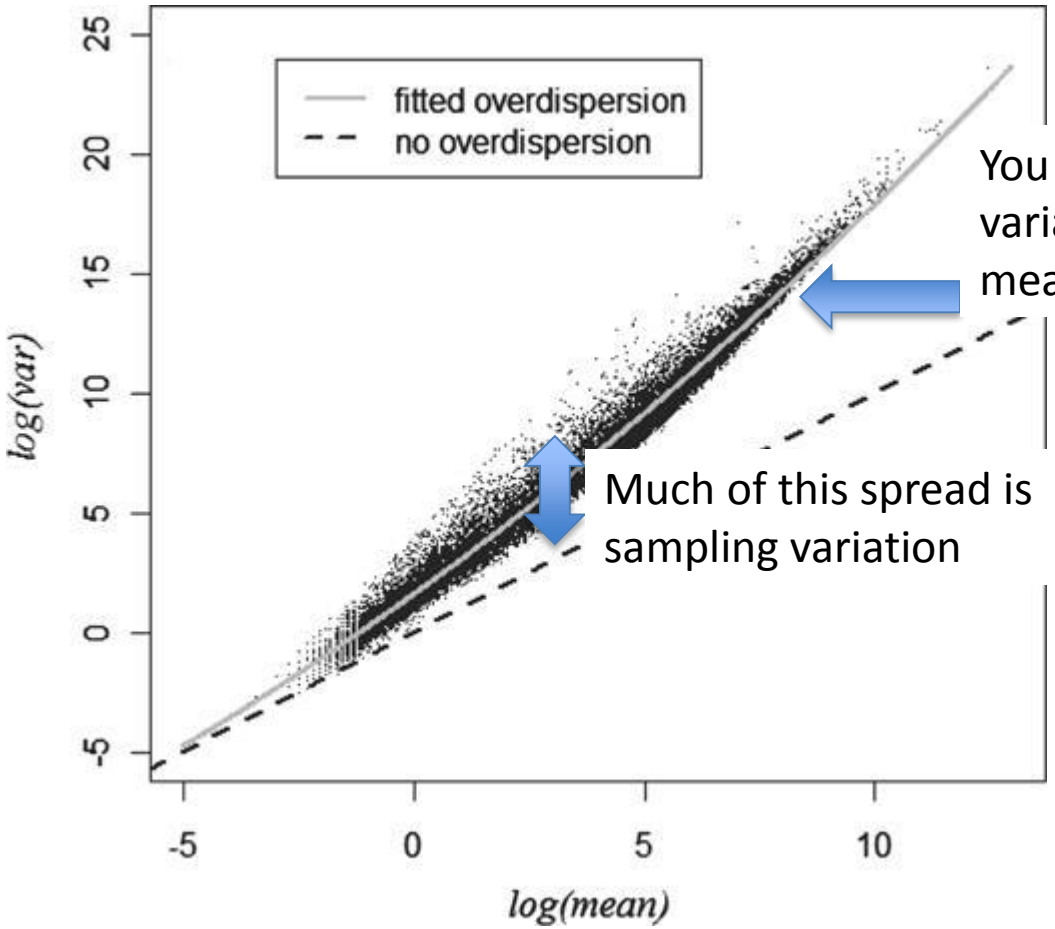
Display total read counts per well in a matrix



Inspection and analysis of control samples



Why differential expression packages provide shrunken estimates of variance to boost power



You can estimate variability from the mean

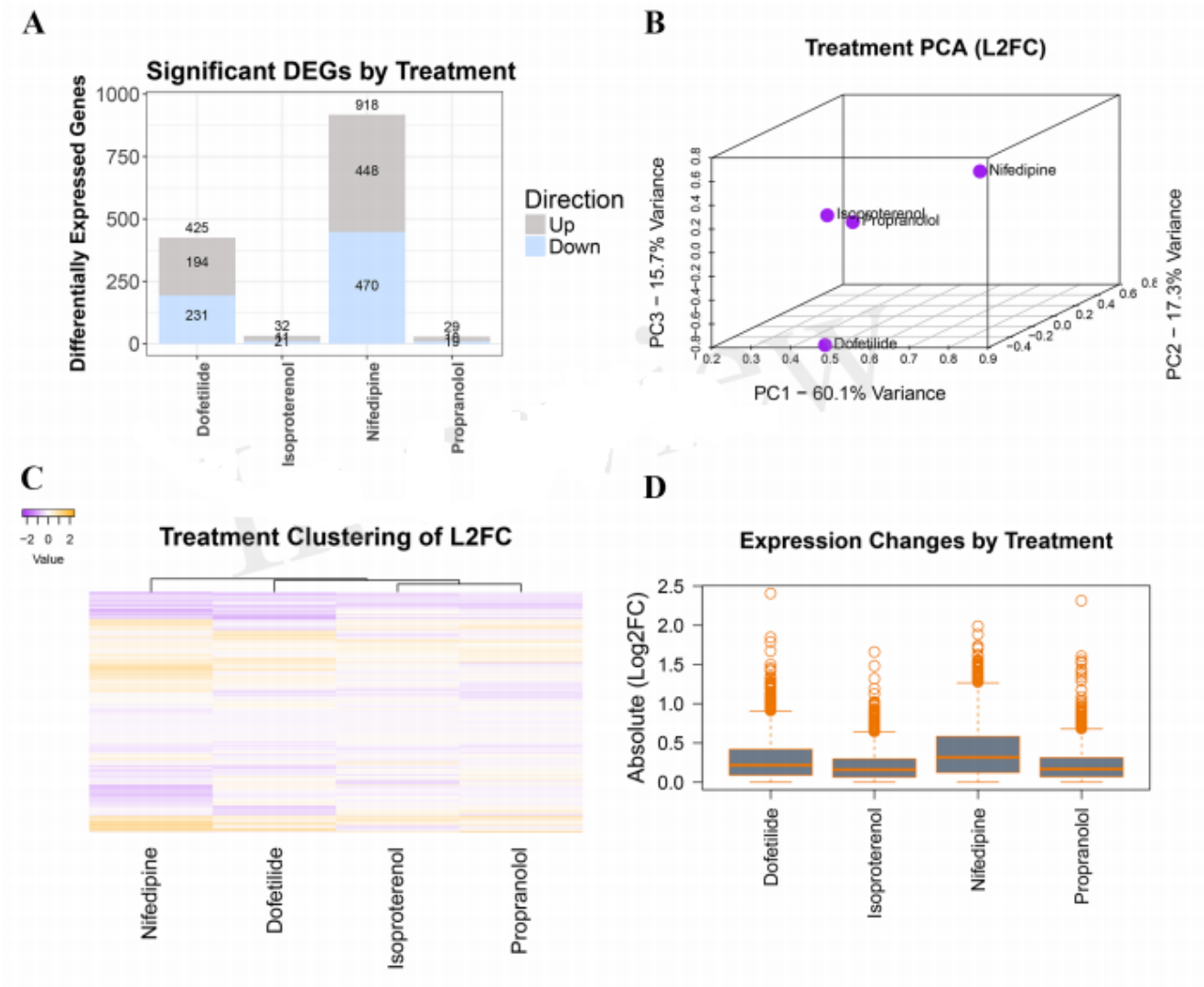
Much of this spread is sampling variation

Zhou et al., *Bioinformatics*, 2011, 27 (19), 2672–2678

The effects of count thresholds per gene

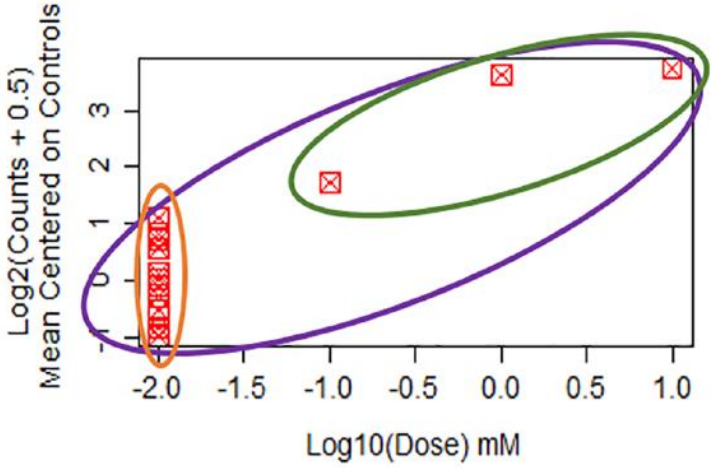
- Set criteria for analysis to be those genes with average counts ≥ 5 (threshold) across samples
- Keep features (genes) with at least ½ treatments that meet criteria
- *qvalue* package to calculate $\hat{\pi}_0$ (estimated proportion of true null genes) by count (%tile)

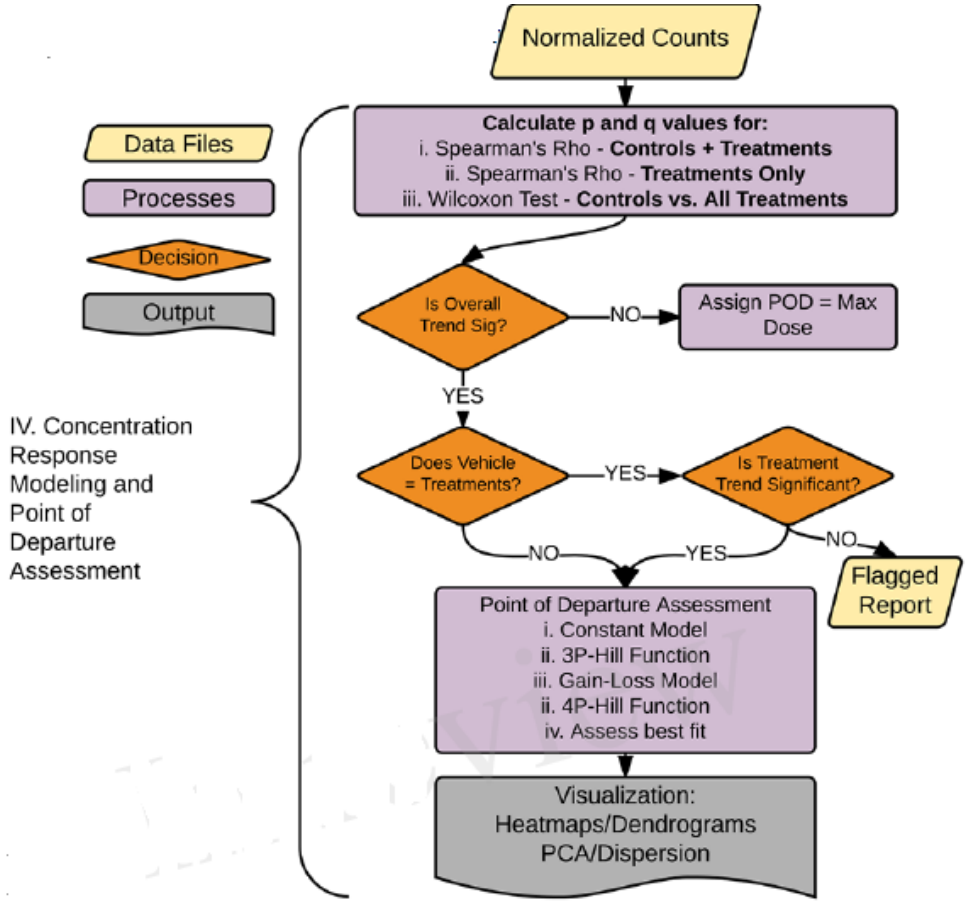
Differential Gene Expression Assessment – 4 chemicals/drugs and treatment of iPSC cardiomyocytes (Rusyn Lab). Analysis by DESeq2



Statistical flag generation and dose-response decision chart

Comparing treatment groups via rank tests and Moment-Corrected Correlation



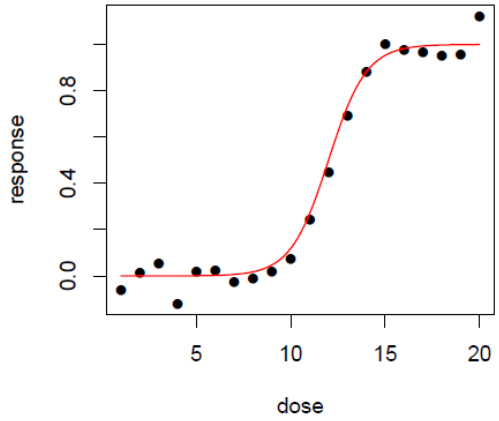


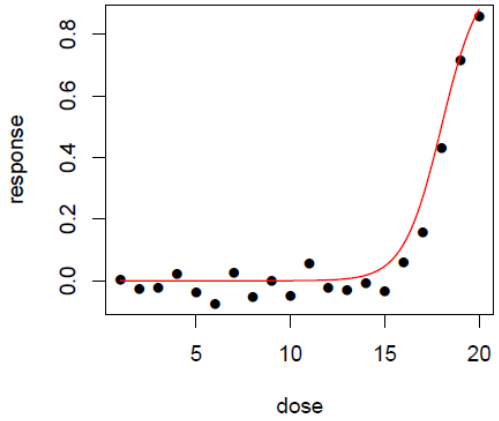
A few remarks on dose-response curve-fitting

- With lots of data, one can explore a large number of models
- With few data points, may need to reduce the number of models explored
- Nonparametric smoothing methods may work okay, but finding appropriate bandwidths may be tricky with little data
- Most points-of-departure involve interpolation, so different reasonable models often agree
- For gene expression, need to handle *testing* as well as estimation

- The 4-parameter logistic model is sigmoidal, has a “floor,” a “ceiling,” and parameters that govern when it rises, and how steeply
- However, depending on the range of doses, the model may offer a reasonable fit to data that might have been modeled more simply

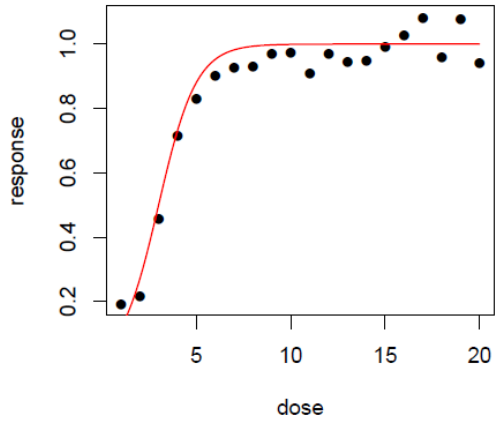
Both floor and ceiling apparent

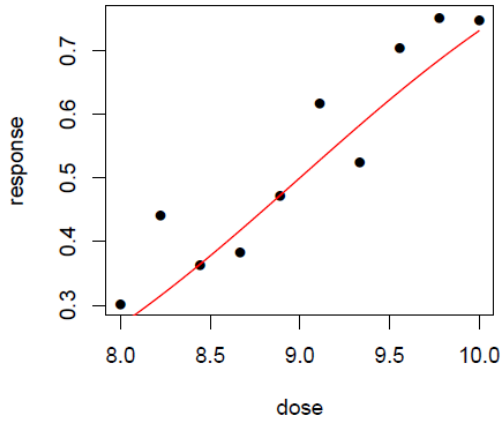




Ceiling not achieved

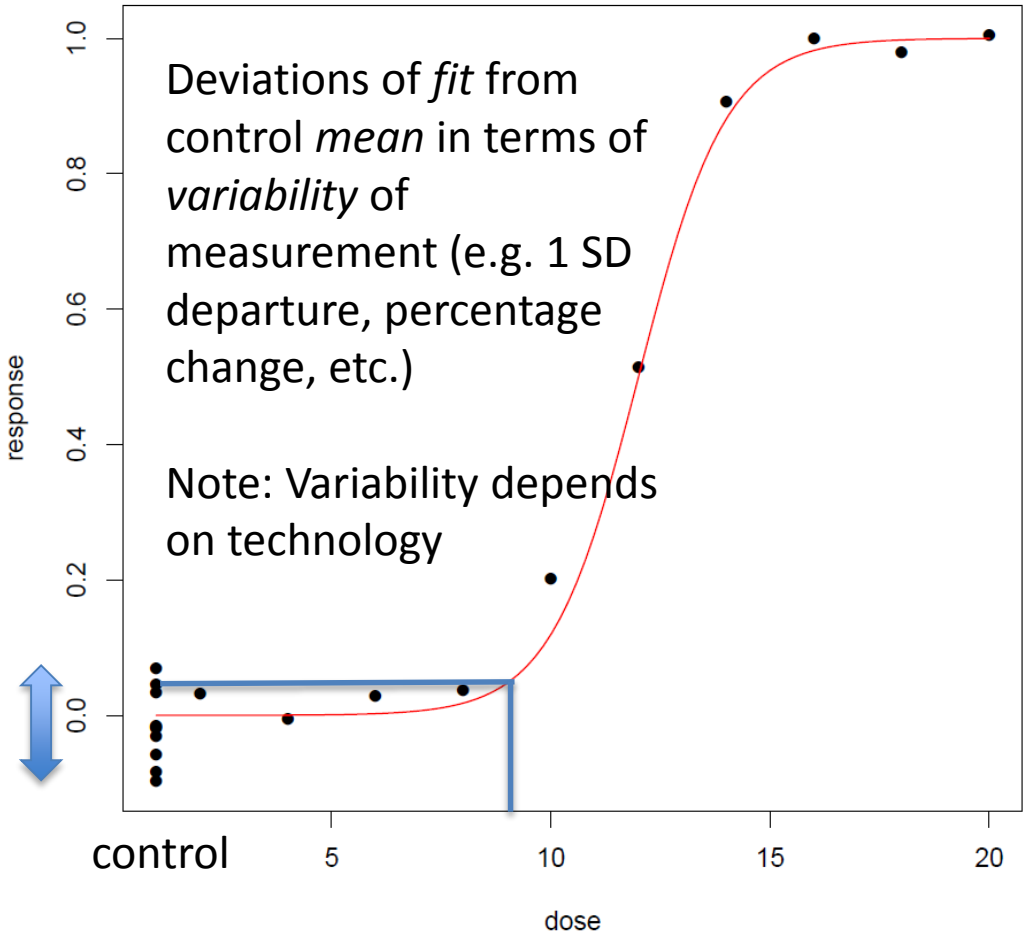
Floor not achieved





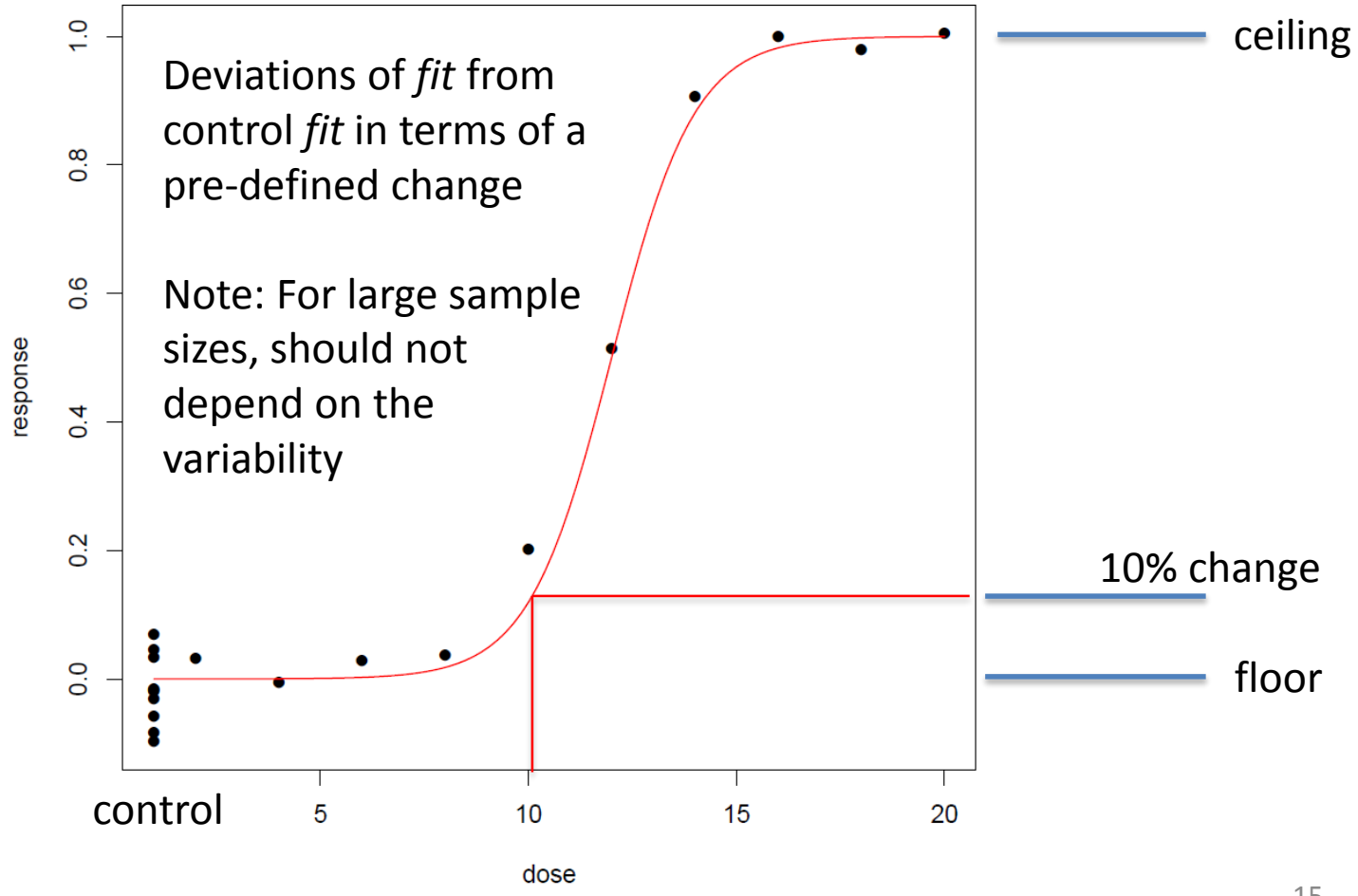
Curve in the range examined looks nearly linear

Benchmark dose typically uses variability to determine points of departure



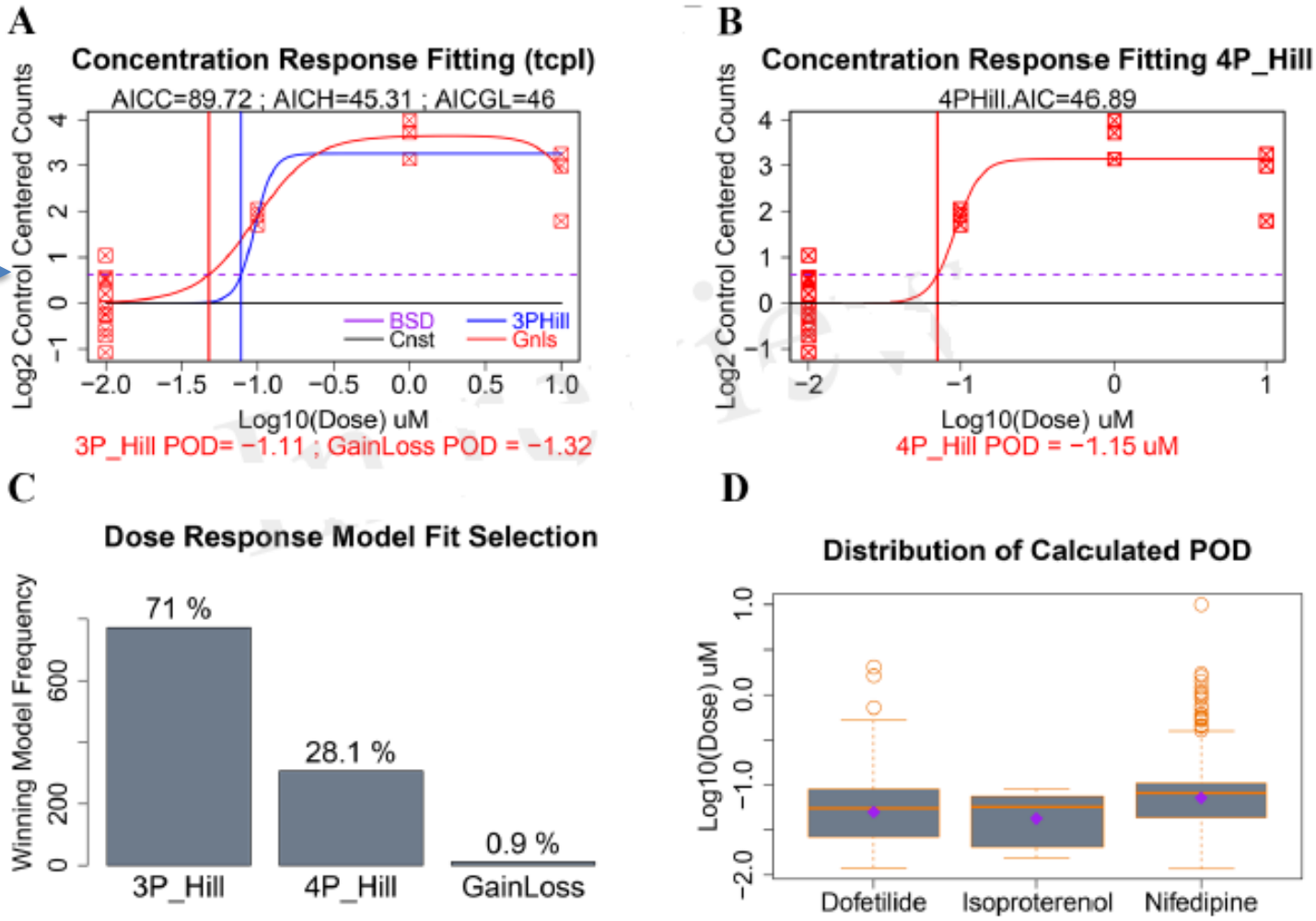
Mean and variance can be computed from controls

EC_{10}, EC_{50} depend on the fit alone



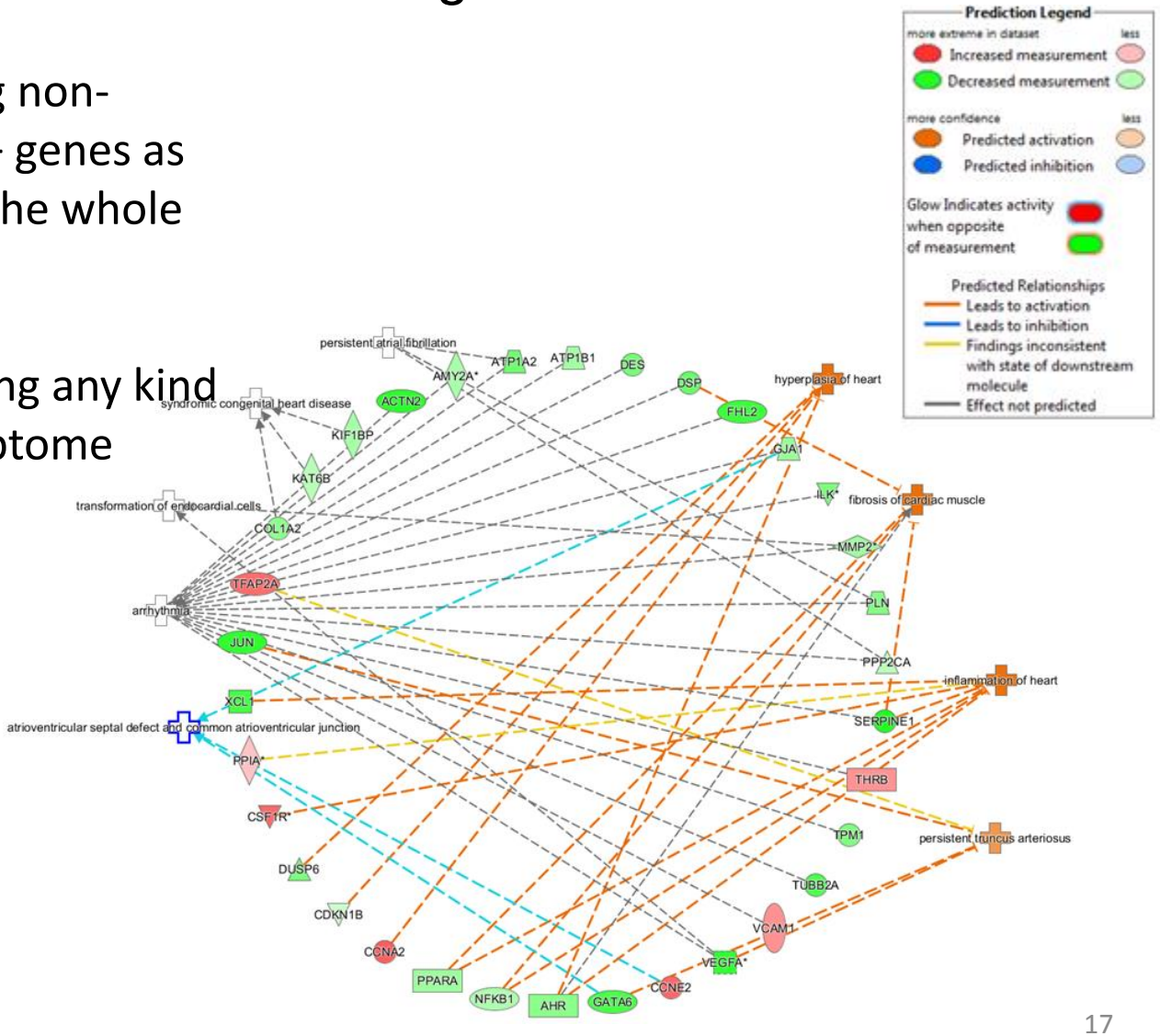
Dose response fitting for the cardiomyocyte data using *tcpl* and *drm* 4P Hill, choose winner based on lowest AIC

POD based on 1 SD departure from control mean



“Significant” cardiac-associated toxicology pathways for dofetilide, based on fold-change

- IPA Analysis, using non-significant S1500+ genes as background (not the whole transcriptome)
- We do not yet using any kind of whole-transcriptome extrapolation



Discovery vs. predictive pathway analysis

- We use simple enrichment approaches (like everyone else), IPA, DAVID/EASE, etc.
- The simple tools provide easy results and some insight
- We and others have critiqued these methods as not providing accurate p-values per pathway, preferring full resampling approaches (e.g. SAFE, GSEA)
- **Final pathway-based PODs are based on minimum median pathway PODs, much like BMDExpress**
- Data on large numbers of chemicals will enable deeper investigations of pathway perturbations, and new methods to fully exploit the data

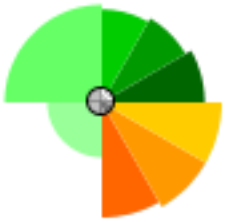
Use of points-of-departure for pathway-based determinations of overall transcriptional POD

- The uncertainty in pathway-based transcriptional points of departure could use further development
- We have been experimenting with bootstrapping to quantify this uncertainty at the per-gene level
- Also, bootstrapping may be very useful to quantify uncertainty for median pathway POD, because the constituent genes are correlated

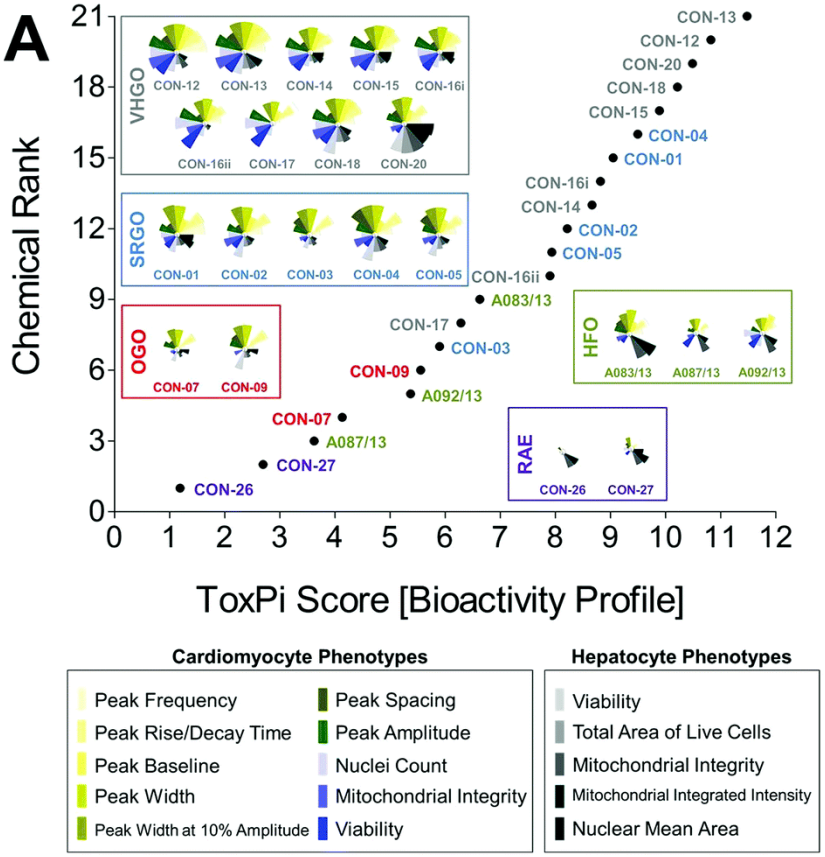
Summary

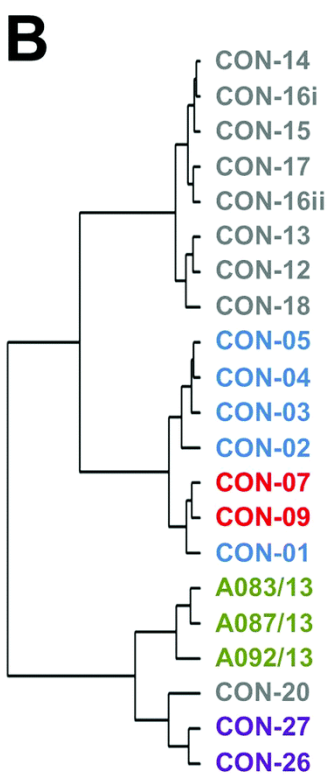
- We have described a pipeline for handling gene expression dose-response data
- Much of the effort concerns the practicalities of QC and handling samples of small to medium size
- Once the foundation is laid, interesting comparisons can be made across multiple chemicals and chemical classes
- For example, I didn't even discuss comparison to databases such as LINCS

Going further 1: ToxPi evaluations of pathway activity



- "slices" are composed similar measured features of possible concern
- Overall ToxPi score reflects weighted sum of slice sizes

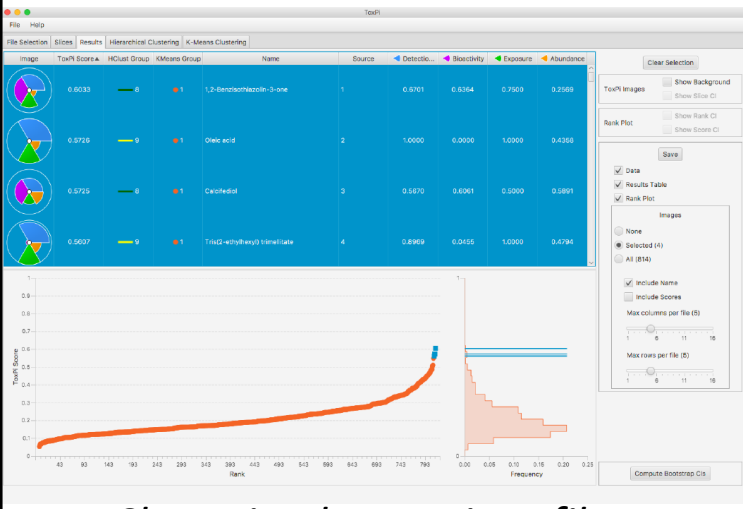




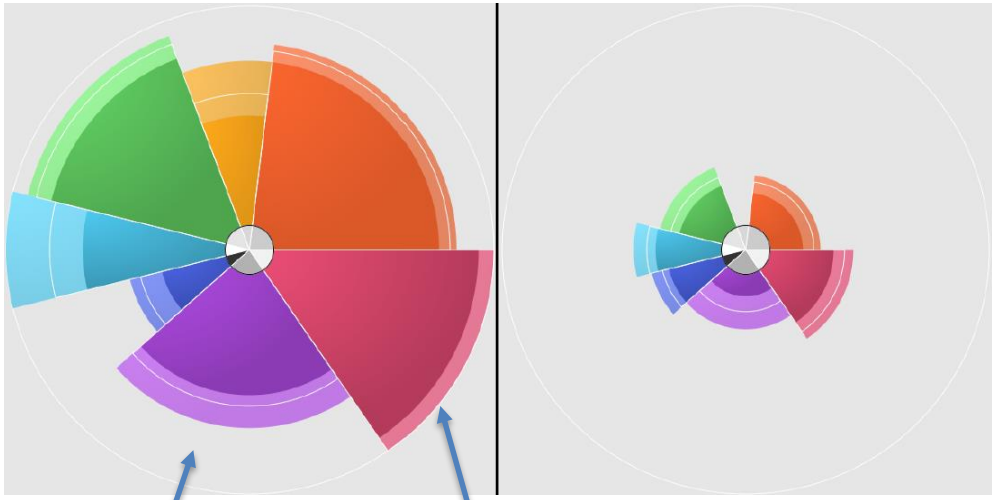
Grim et al., *Green Chem.*, 2016, 18, 4407-4419

Going further 1: ToxPi evaluations of pathway activity (ToxPi 2.0)

Updated interface



Slices and uncertainty



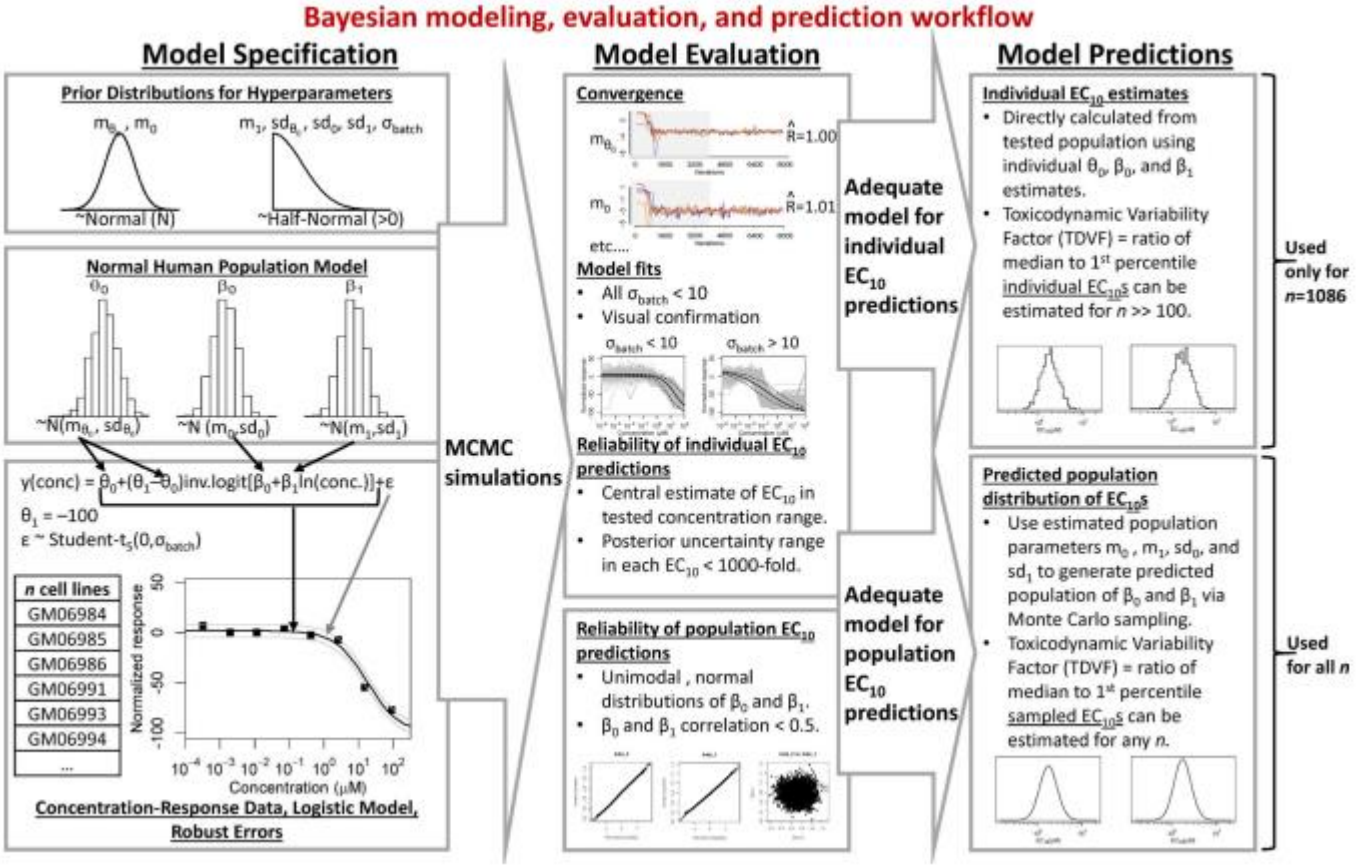
Clustering by ToxPi profile

PODs of genes in expression pathway 1

PODs of genes in expression pathway 2

Going further 2: Evaluating population variability in pathway response (human cell line studies, mouse studies)

Workflow for estimating underlying variability when *n* samples measure a quantity with error (Chiu et al., ALTEX. 2017 ; 34(3): 377–388. doi:10.14573/altex.1608251)



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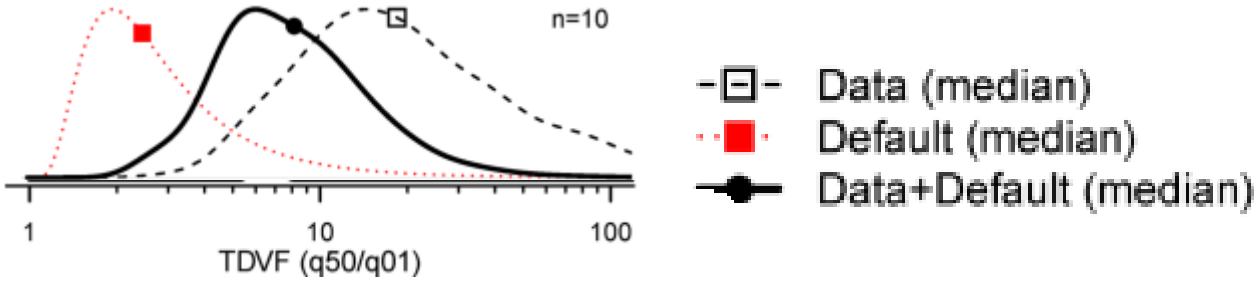

- Can we (should we) be doing this analysis for gene expression pathway PODs?
- (Otherwise, when hundreds/thousands of chemicals are calculated, the most extreme-appearing will be over/under-estimated)
- How to approach it?

Acknowledgments

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