

Compound Ranking with Low-Replication High-Throughput Assays: A Basic Guide for Practitioners

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Early Clinical Development

 **Pfizer** WORLDWIDE RESEARCH & DEVELOPMENT

 **SCIENCE**
FOR LIFE-CHANGING
IMPACT 

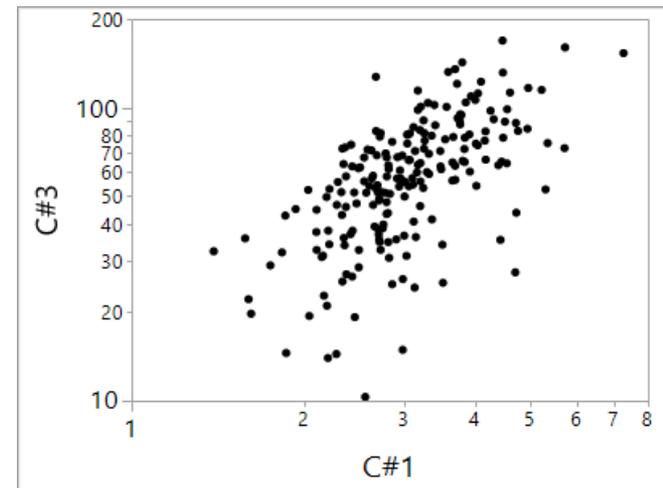
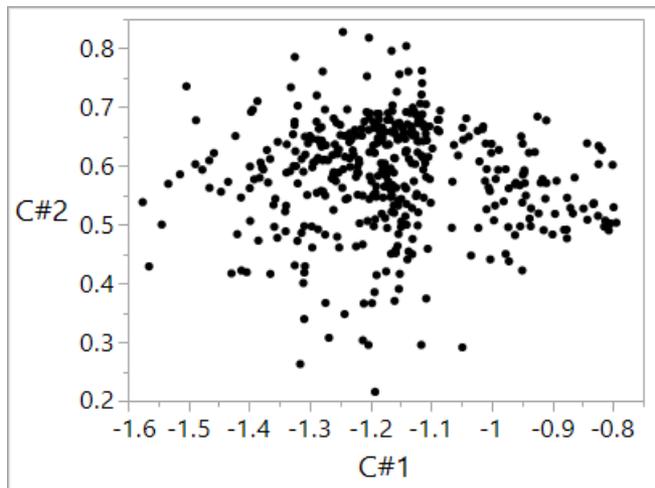
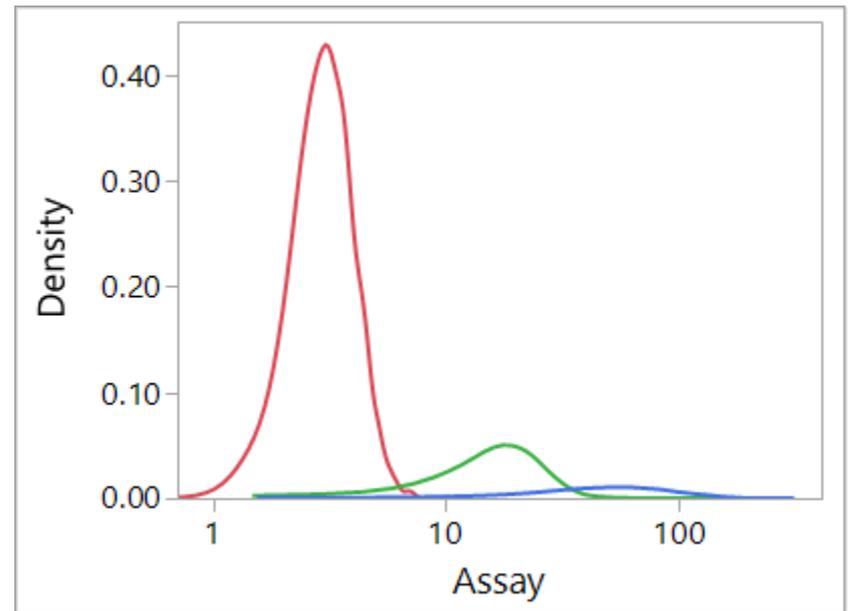
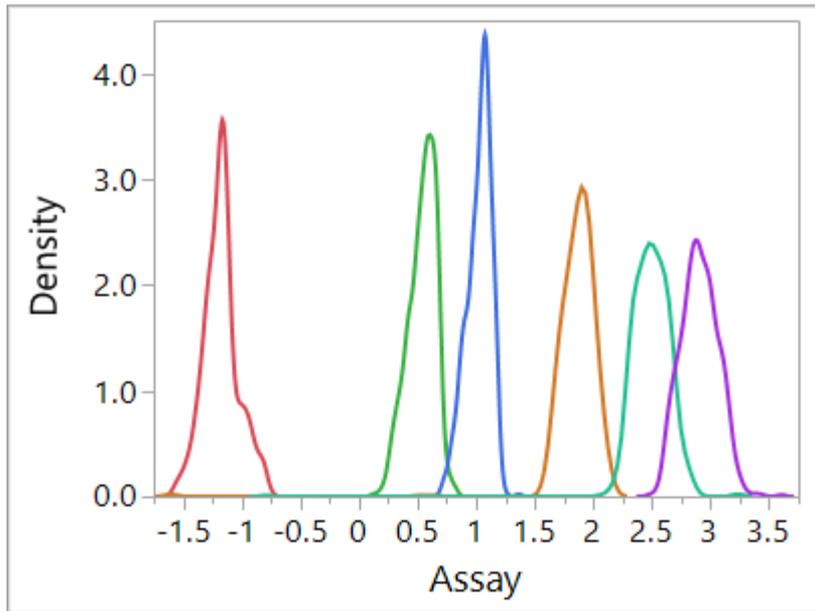
Opening Remarks

- Ranking Chemical Matter is Ubiquitous in Drug Development
- High-Throughput Screening
 - Activity Assay, Large No. of Molecules Screened, Threshold Applied
- Lead Development and Optimization, Compound Selection
 - Looking for Molecules with Desirable ADME Properties
 - Solubility, Clearance, Permeability, Safety Liability
 - Handful to Hundreds of Compounds
 - Most Molecules Measured Once (perhaps Twice)
 - Biochemical and Cell-Based Single Endpoint Assays are Common
 - Approaches to Ranking
 - Sort Low-to-High, Binning, Graphs with Multiple Dimensions, Multi-Objective Methods (e.g., Desirability Functions), etc.

Statistical Considerations and Approach

- Ranking and Statistics
 - Focus on Estimation (vs. Hypotheses)
 - Multiple Comparisons, Joint RVs, Order Statistics, Stochastic Ordering, Agreement, Intra-class Correlation, Equivalence, etc.
 - What's Big?
 - Chemist vs. Biologist View, Within X-Fold, <10 10 - 30 >30
- Tactic Employed Here
 - Historical Assay Serves as Springboard
 - Simulate (and Simulate) Use Cases
 - Emphasize Skewed Distributions
- Not Addressed
 - Assay Drift/Shift, Dose Response Curves/IC₅₀-Shift, Partial or Incomplete Ordering, Predictive Models/Binning

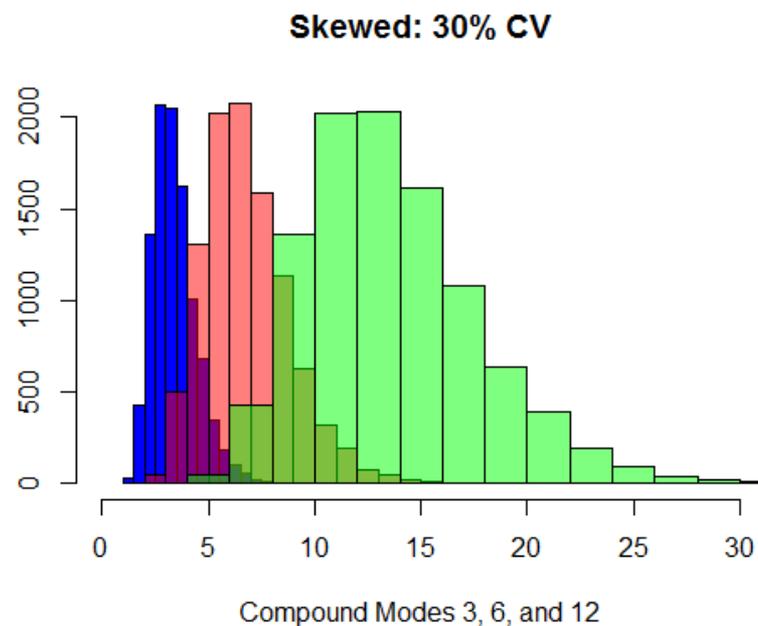
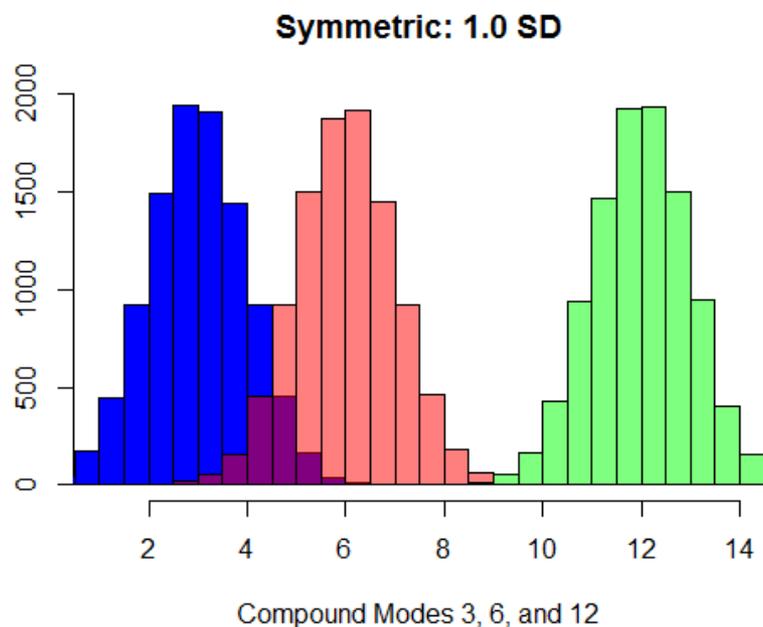
Chemical or Cell-Based Assay?



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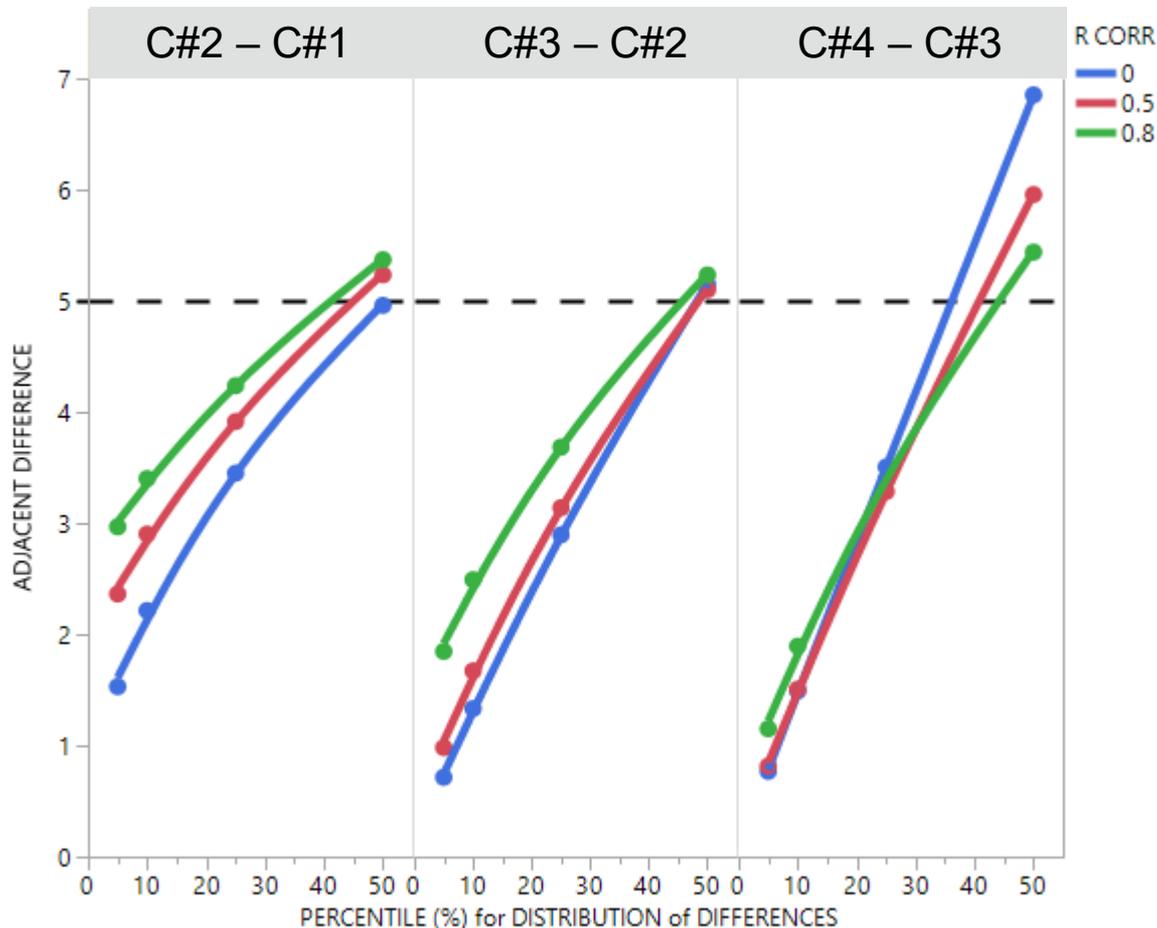
Symmetry or Skew: ~ 30% Coefficient of Variation (CV)

Distribution Centers at 3, 6, and 12 : Average Range is 4-Fold

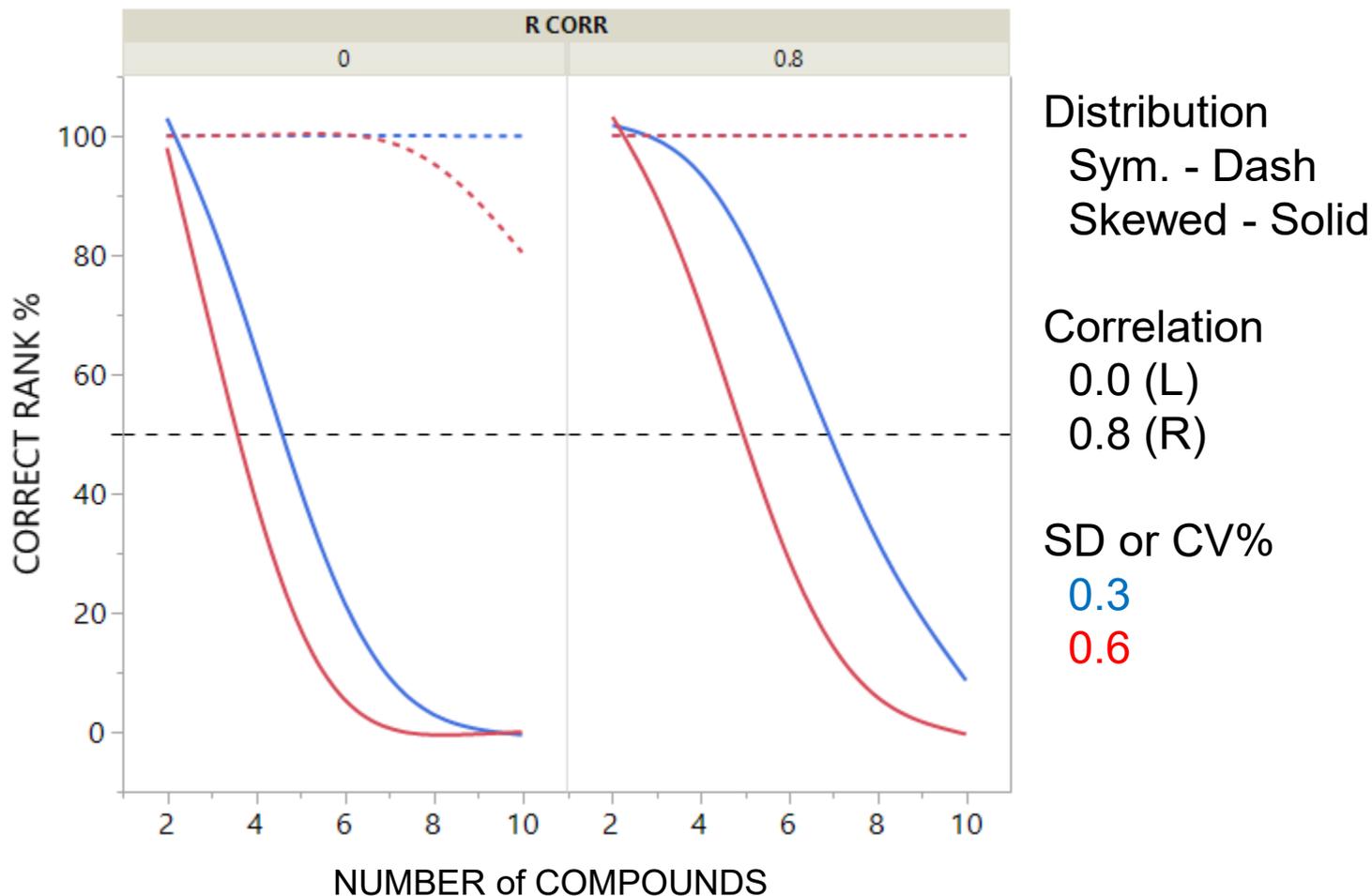


Adjacent Differences for Correctly Ranked Samples

- 4 Equal-Spaced Molecules at $3_{C\#1}$, $8_{C\#2}$, $13_{C\#3}$, and $18_{C\#4}$
 - 1 Replicate, Skewed, 30% CV, Intra-class Corr. 0.0, 0.5, and 0.8



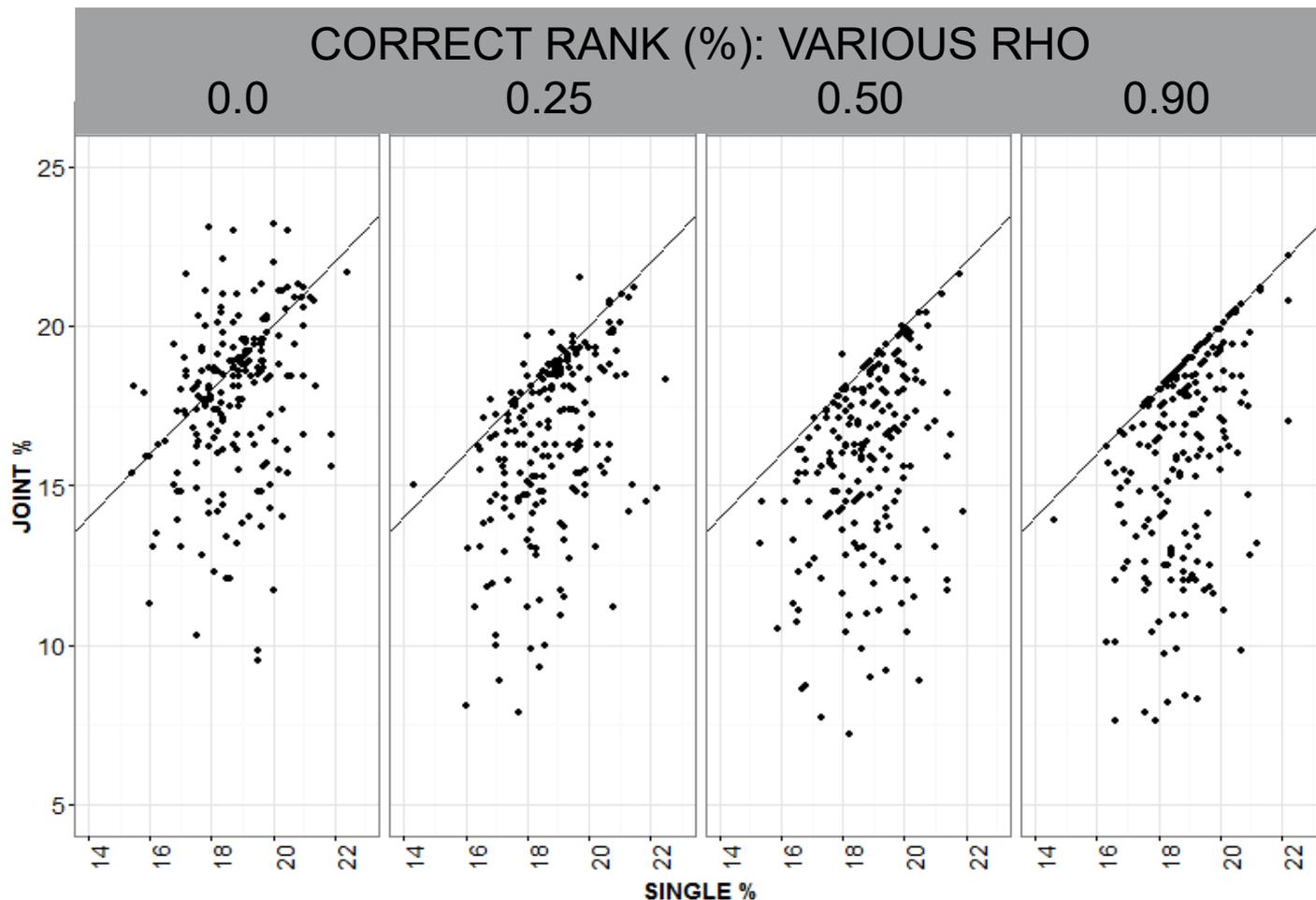
Rank Performance for Equally Spaced Molecules on [3, 18]



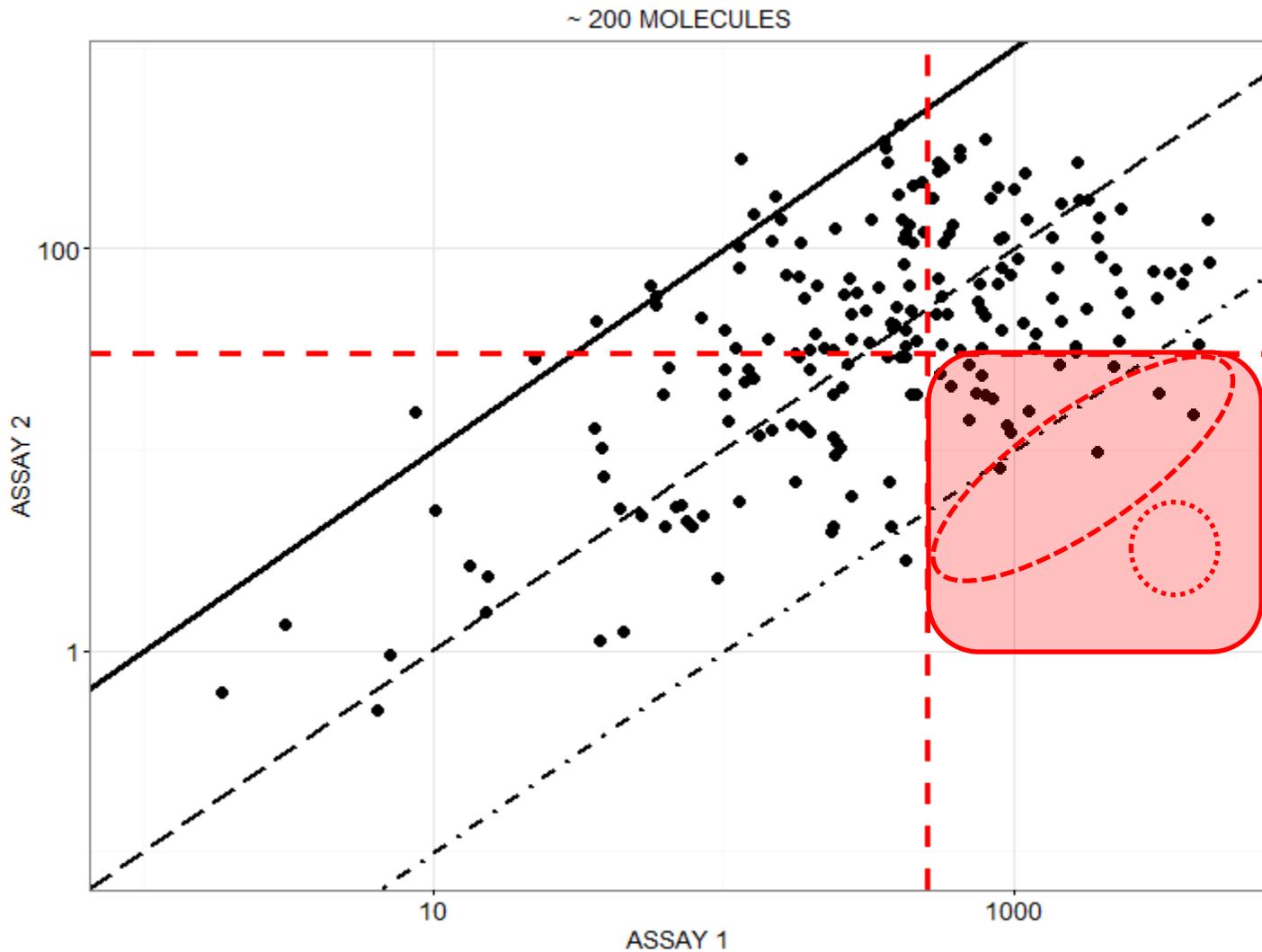
Complement 1st Assay with 2nd Positive Correlated Assay

1st: 6 Molecules, [3,18], 30% CV
'Higher is Better' Desirability Function

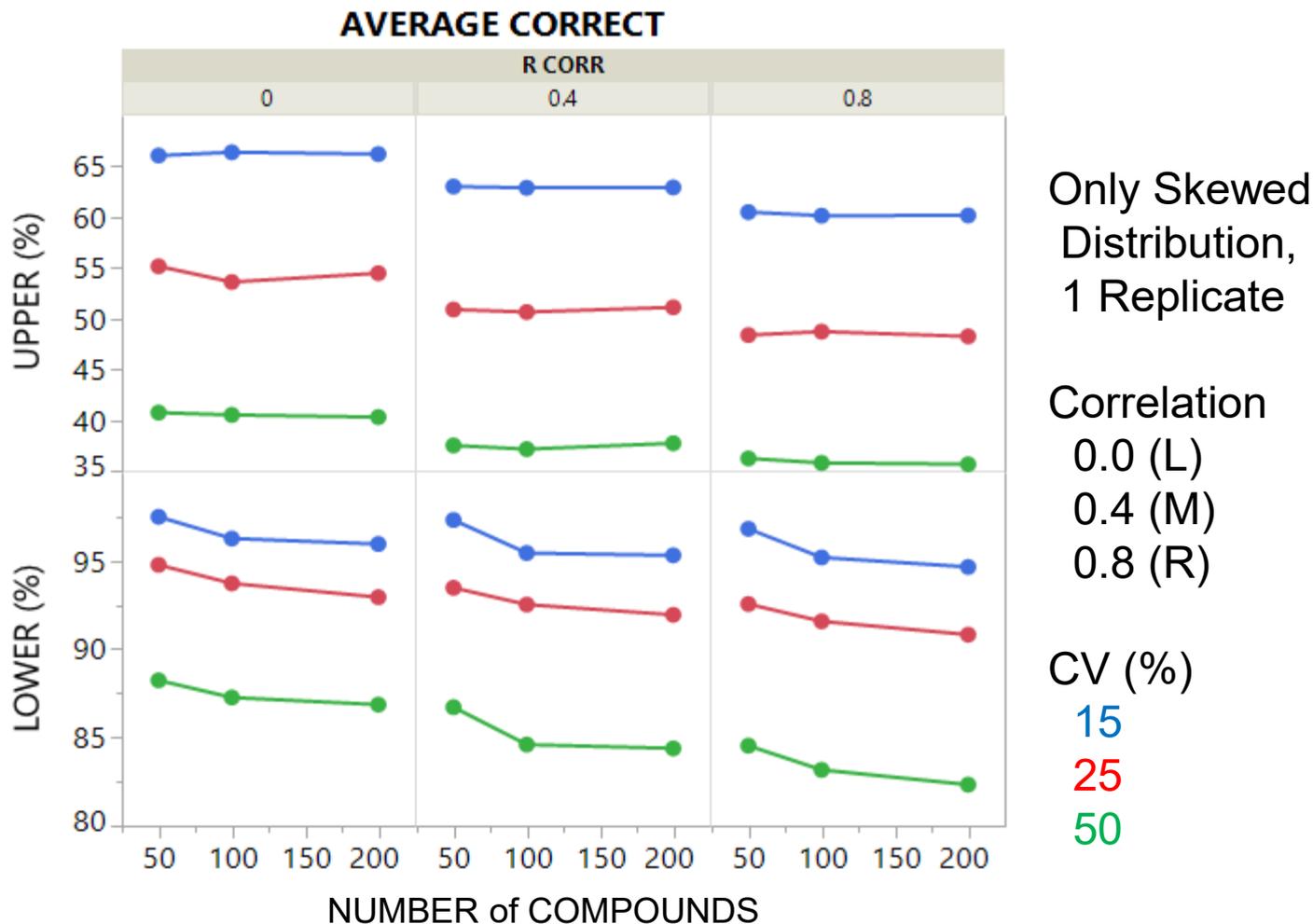
2nd: Assay Spans 4-log Range, 15% CV



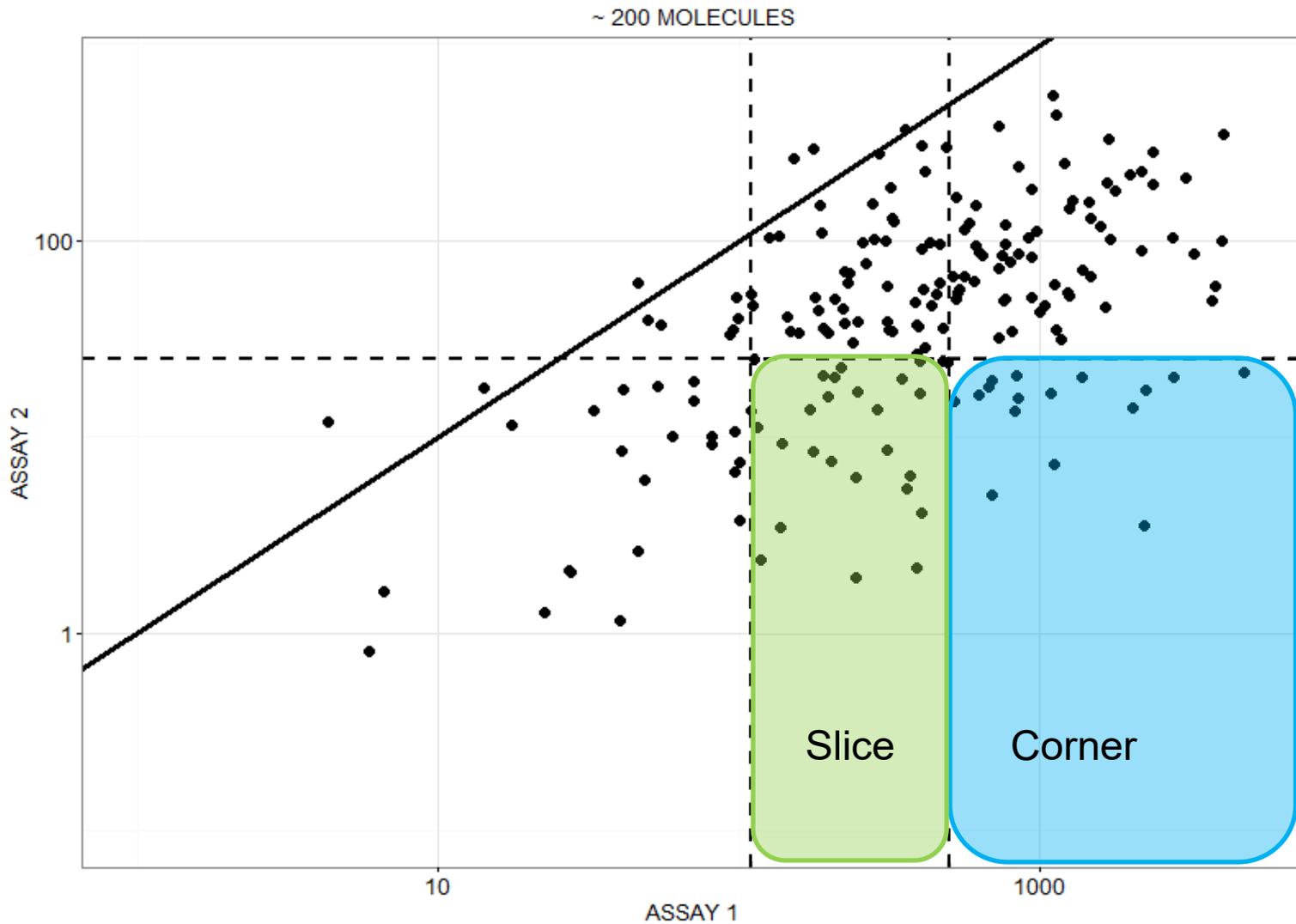
Multiple Assays for Each Molecule: Real Line ↗ Plane



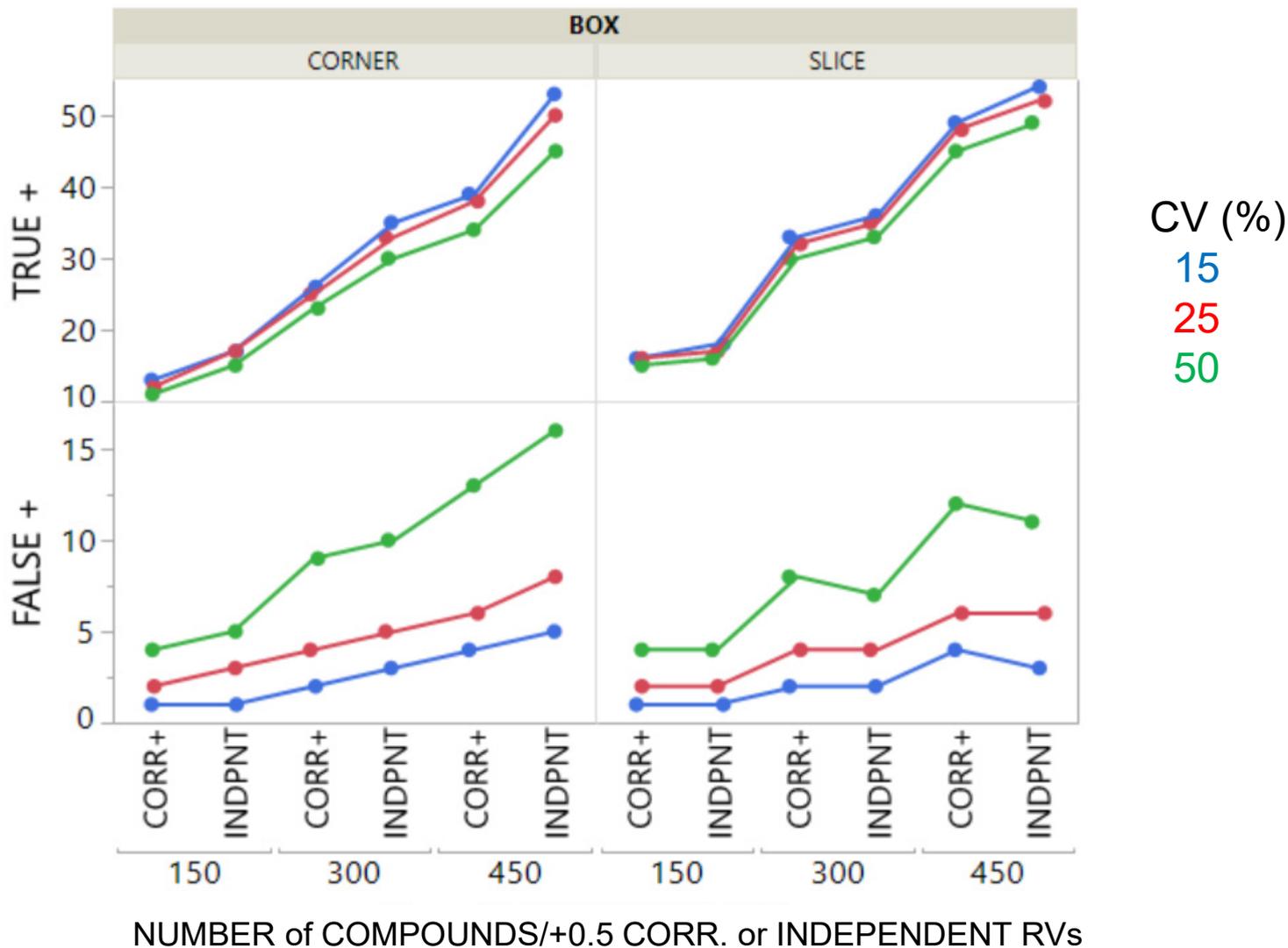
Partial Ranking: % Correctly Contained in Upper and Lower 10% Tail for Two Assays



Fencing Helped Conquer the West, Right?



Fence Hopping - Average True and False Positive Counts



Practical Guidance – One Dimension

- Ranking Random Variable Observations is Not Easy
 - Simulation is Helpful for Practical Use Cases
- Some Recommendations on \mathbb{R}^1
 - Idea of Assay Distribution for One Molecule, e.g., Skew, Std Dev or %CV, $SD \propto \mu$
 - Intra- and Inter-Experiment Behavior for Various Assays
 - Limited Practical Role for Replication and Tweaking Summary Stats
 - Value of Moving Toward Binary 1-0 Discrimination
 - Sorting 50 Compounds on 4-Fold Range is Foolhardy
 - Boundaries are Blurry
 - Distribution for Population of Molecules
 - ‘Controls’ are Buoys Bobbing Around in a Sea of Unknowns
 - Question Value of Strict Reductionist Approaches

Practical Guidance – Multiple Dimensions

- Recommendations on \mathbb{R}^2 (+ \mathbb{R}^n)
 - Difficult Problem
 - Can be Bounded Above by Single Assay Results
 - Range of Desirability or Fuzzy Loss Functions Possible
 - Complementary Assays Build Confidence (vs. Improve Ranking)
 - Binning/Boxing is Not Going Away
 - Boundaries Should Be Imprecise (Grade Some Molecules on a Curve)
 - Tolerate False Positives
 - Positional Awareness, i.e., Center or Tail of Distribution, No-Man's-Land
 - Applicable Intuition
 - Generally, More Variation is Bad
 - Correlated Outcomes Carry Momentum

Thank You