Novel Protein-Protein Interaction Assays for Human Androgen and Estrogen Receptors

Metabolic Retrofit of Androgen Receptor Assay with mRNA Transfection

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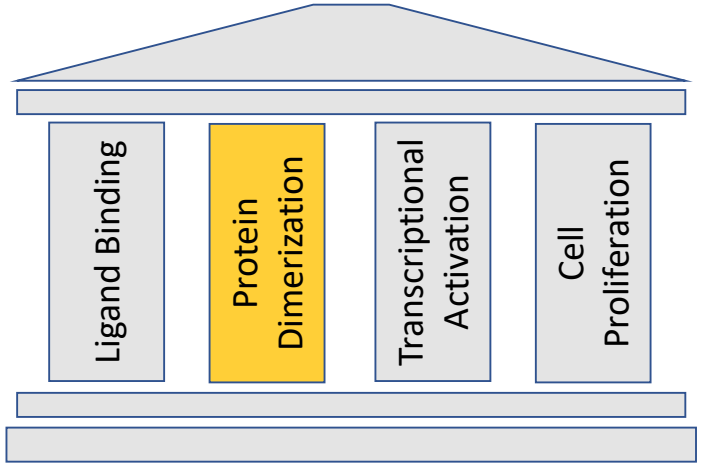
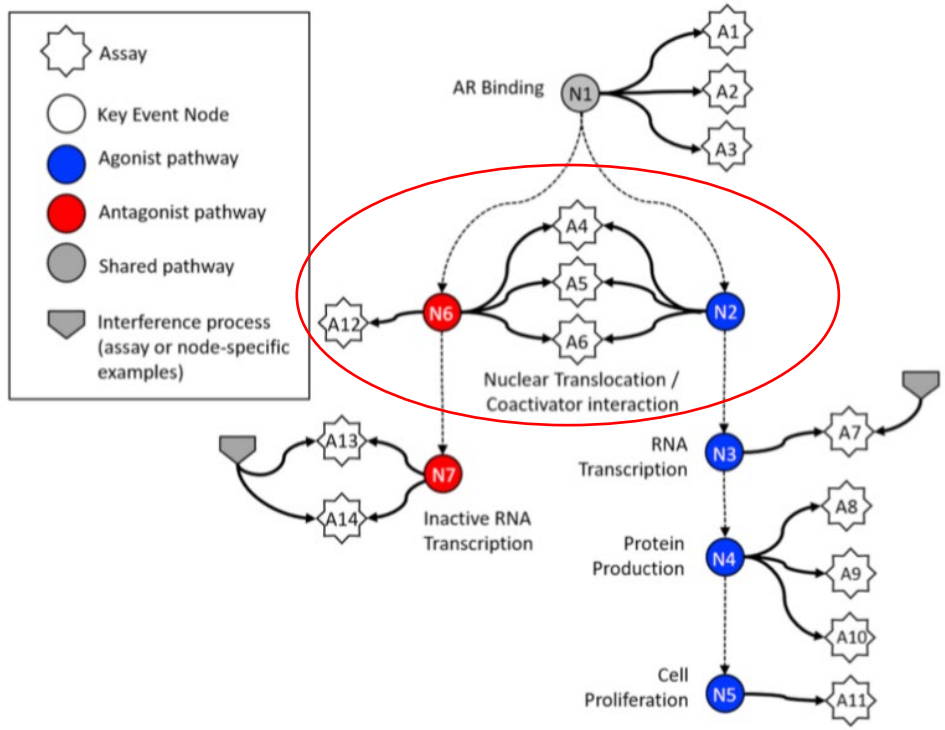
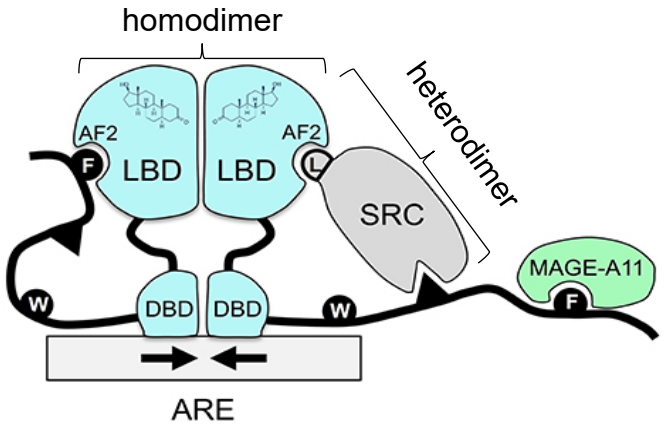
Protein-Protein Interactions Are Indispensable to the ER and AR Predictive Models



Judson *et al.*, 2015 PMID: 26272952
Judson *et al.*, 2017 PMID: 28993267

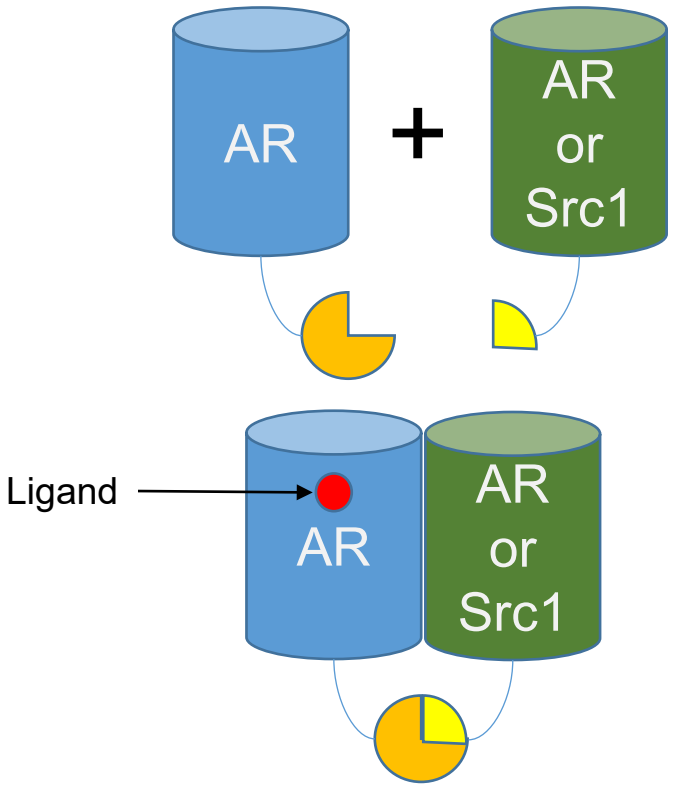


Judson *et al.*, 2020
PMID: 32798611

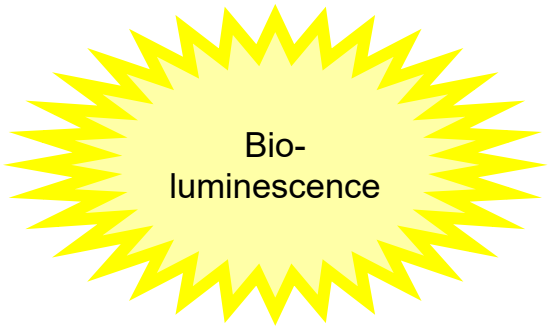


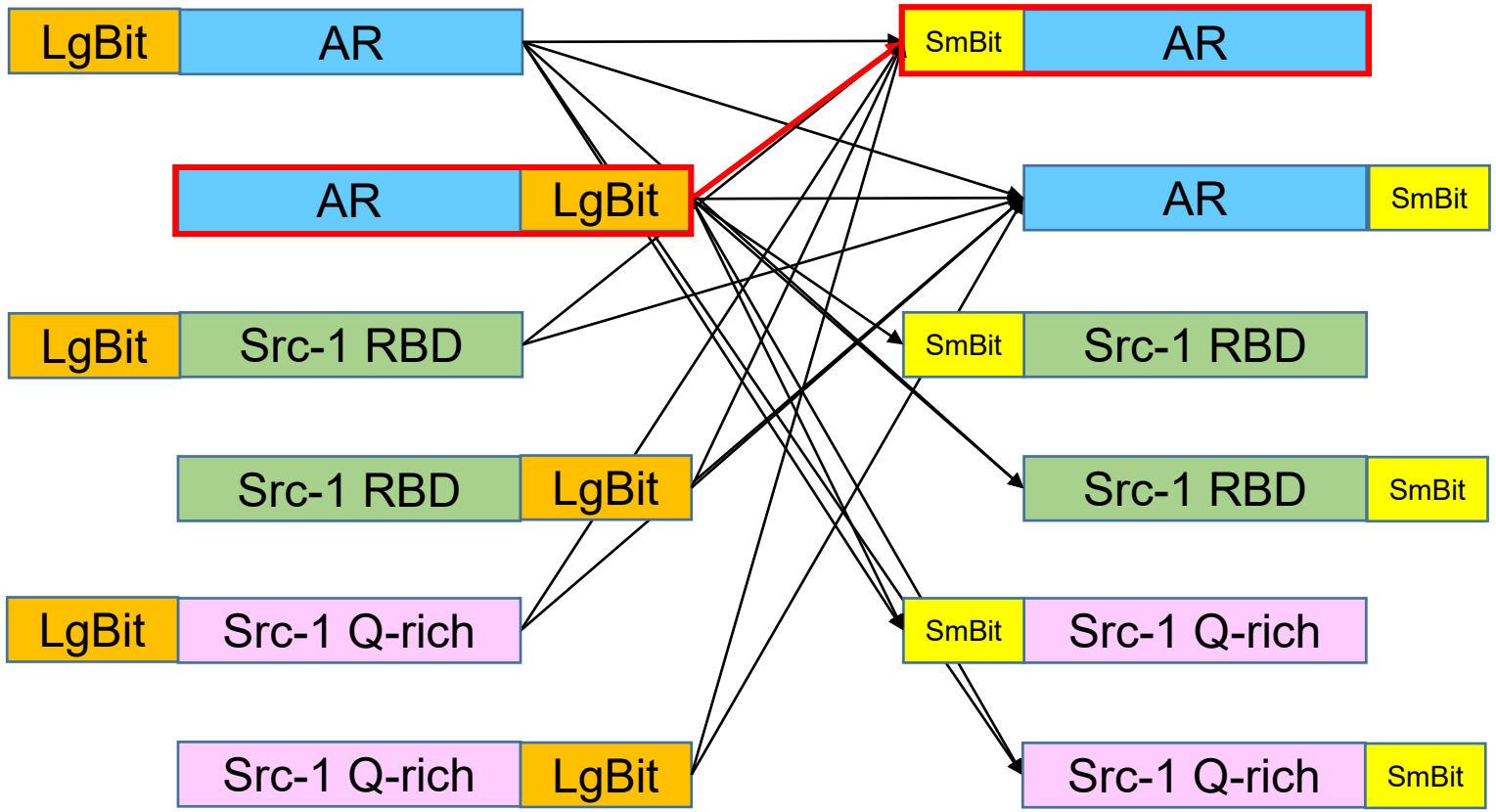
- Nuclear receptors including ER and AR are both receptors *and* transcription factors
- The ER and AR Predictive Models rely upon assays that measure each of these key functions
- EPA needed to develop new assays to measure ER and AR protein-protein interactions

Protein-Protein Interaction Assays Using NanoBit Technology



+ NanoLuc substrate





- Process repeated for both ER-α and ER-β

AR2 Assay Workflow

Plate Cells
(Certus)

24
hr

Test Chemicals
(Echo)

18
hr

Furimazine and Alamar Blue
(Certus)

NanoLuc Detection
(Clariostar)

1
hr

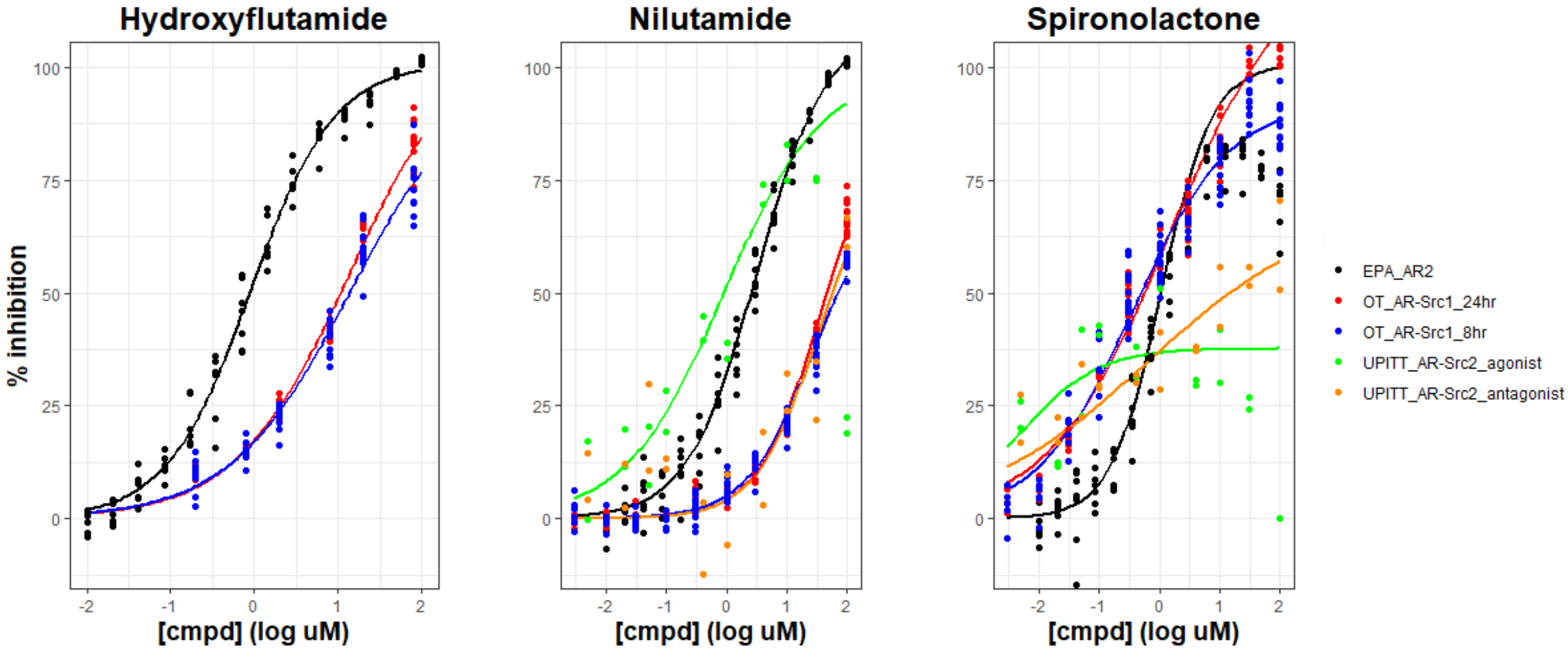
Cell Viability
(Clariostar)

Certus Leap: pressurized, multi-channel solenoid dispenser

Labcyte Echo 555: acoustic liquid dispenser

BMG Clariostar: multimode platereader

AR2 Assay versus Existing AR Antagonist Assays: Reference Chemical Study



- Using a pre-optimized protocol, the AR2 assay correctly identified reference AR antagonists
- Potencies and efficacies in AR2 assay were ≥ those for existing assays for most reference chemicals

Metabolic Retrofitting with Human CYP450 mRNAs

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J Pharmacol Toxicol Methods. 2018 ; 92: 77–94. doi:10.1016/j.vascn.2018.03.002.

mRNA Transfection Retrofits Cell-based Assays with Xenobiotic Metabolism

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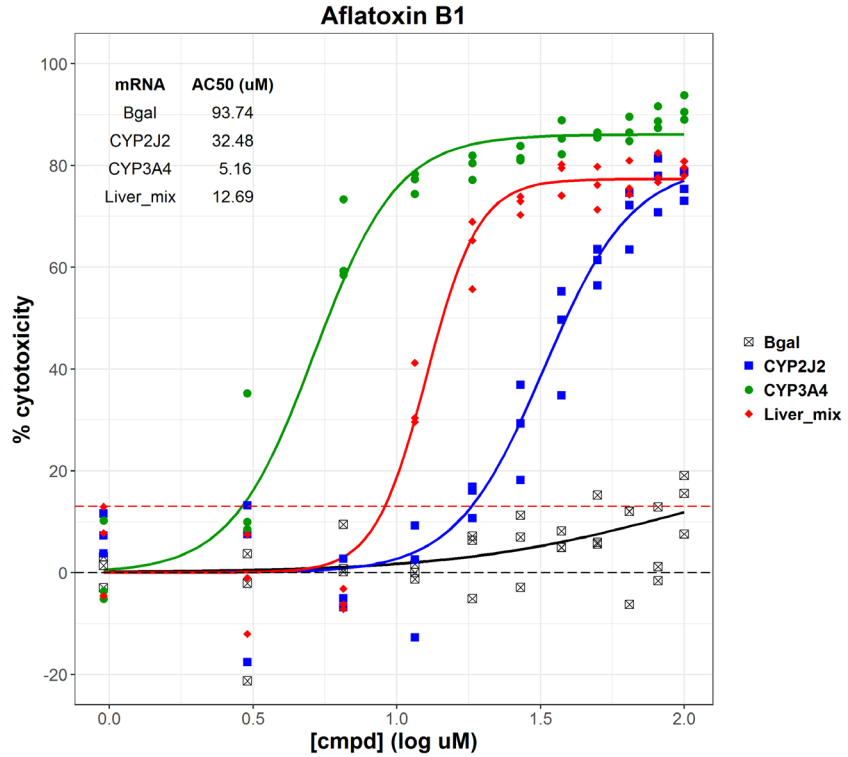
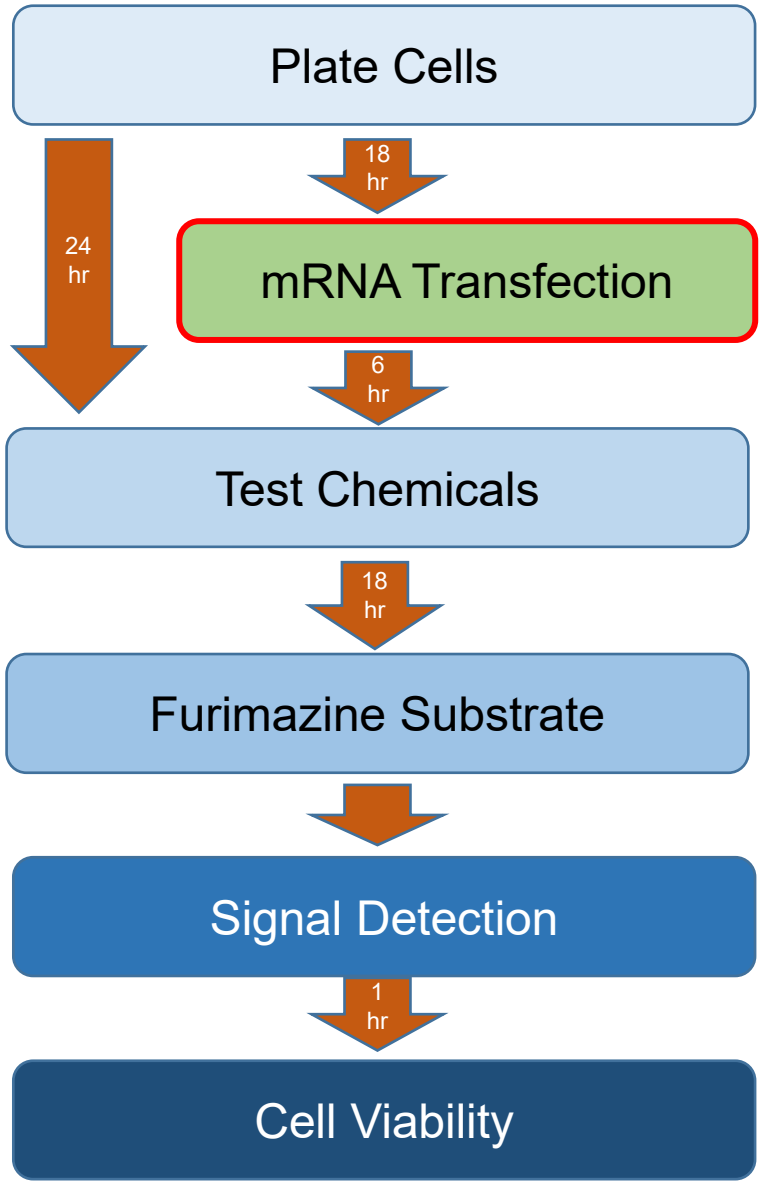
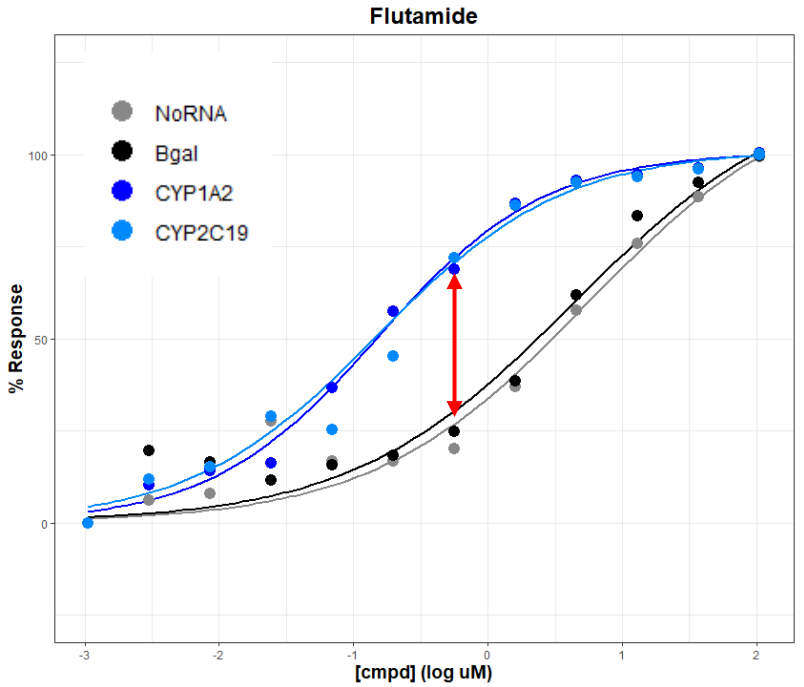


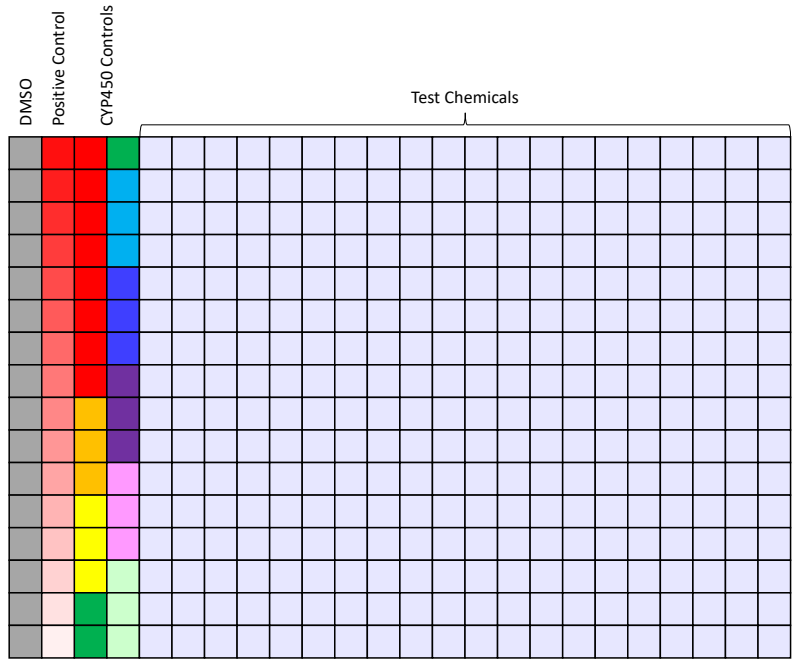
Study Highlights

- **mRNA Transfection:** Bypasses transcription and permits use-defined co-expression of multiple gene products
- **Modified mRNAs:** Chemically-modified nucleotides and 5’ caps reduce RNA immunogenicity
- **Fast:** Steady state CYP450 activity within 8-16 hours
- **Inexpensive:** cost of € 0.05 ($0.06) per well in 384-well format
- **Human metabolism:** Uses the top 10 CYP450 enzymes found in human liver
- **Effective:** CYP450 altered bioactivity observed even under standard HTS conditions (0.5% DMSO)
- **Portable:** Can be performed in most labs- no special equipment required
- **Complete Methodology**: Walk-through on how to characterize and optimize CYP450 enzyme expression in a new cell line

Retrofitting the AR2 Assay with Human CYP450 Activity







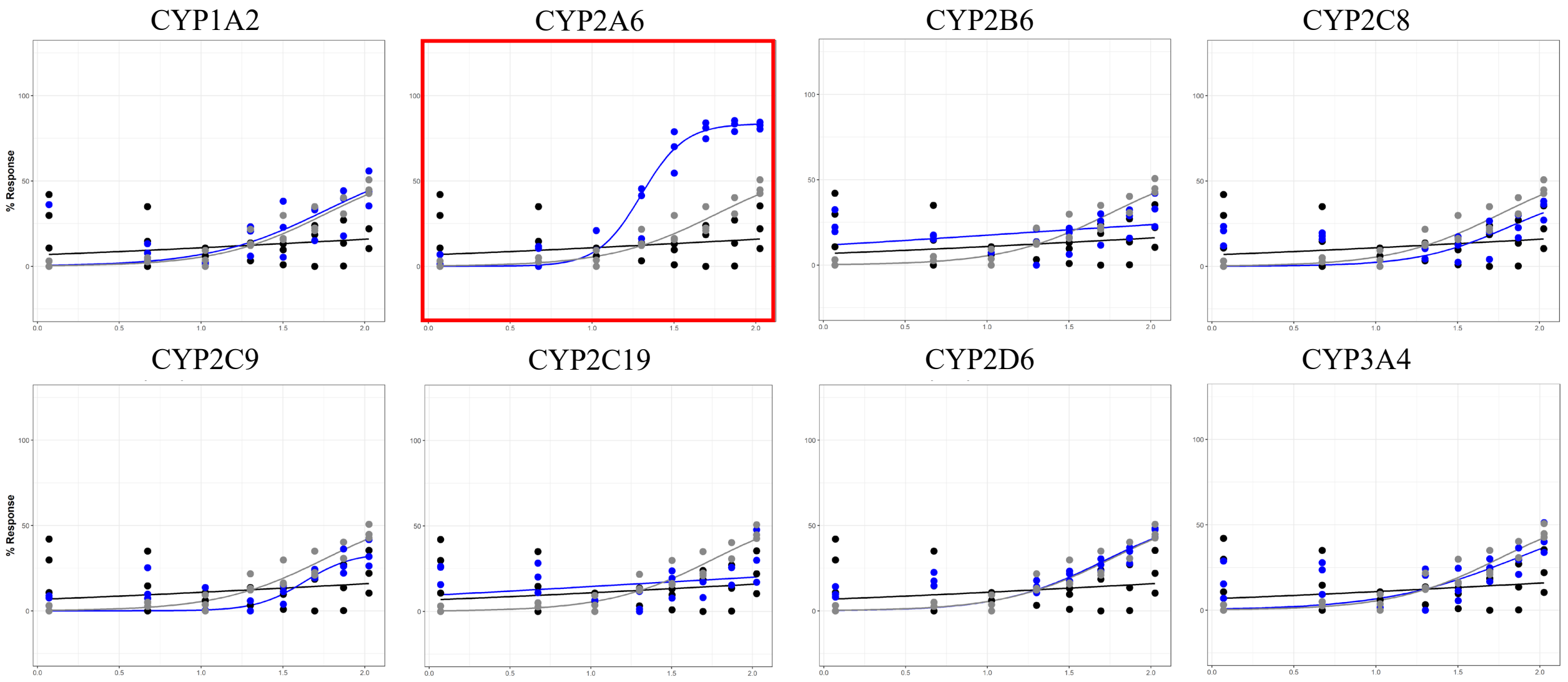
Screening Plate Design

CYP450	Control
CYP1A2	Flutamide
CYP2A6	?
CYP2B6	?
CYP2C8	?
CYP2C9	?
CYP2C19	Flutamide
CYP2D6	?
CYP2E1	?
CYP2J2	?
CYP3A4	?

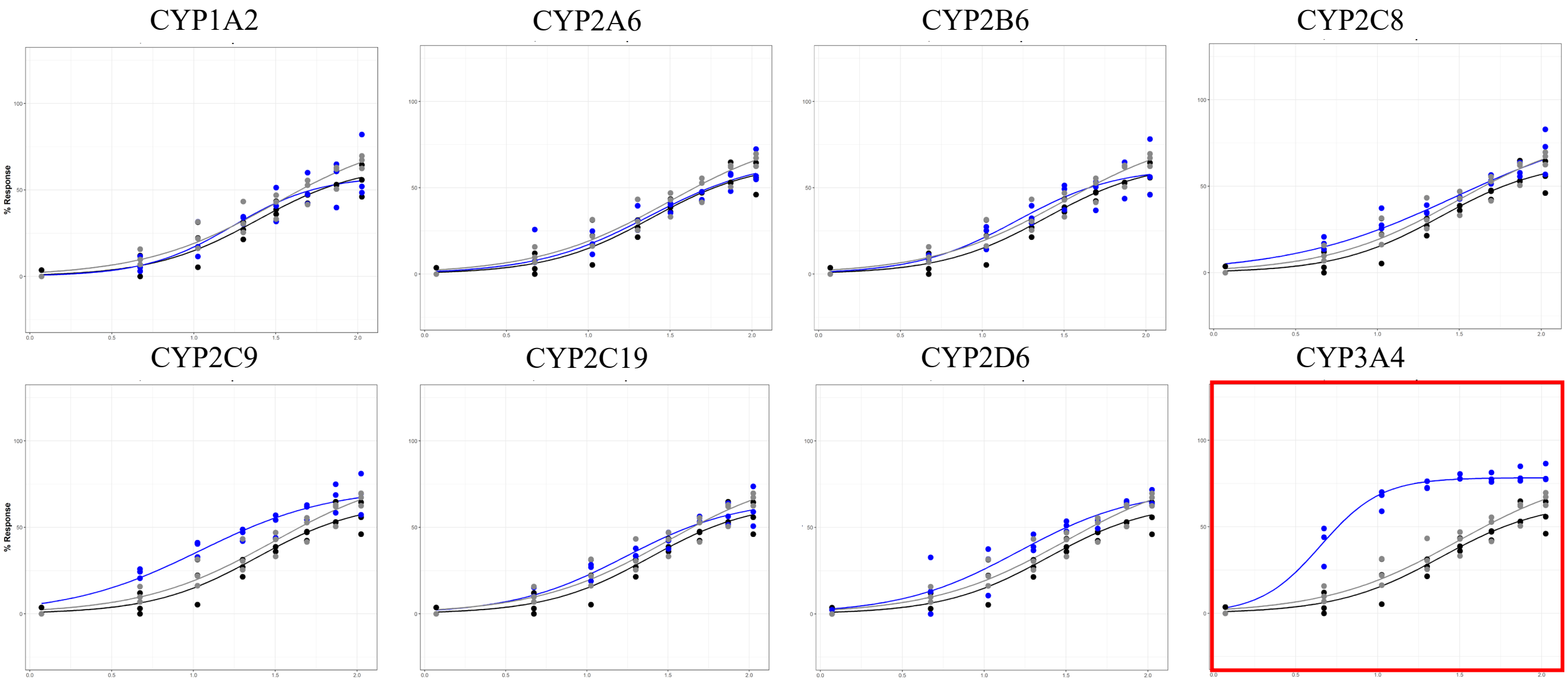
Candidate Search: Screened 960 chemical samples (final 0.5% DMSO) using tiered approach to identify at least one candidate for each CYP450 enzyme

Follow-Up: Identified 30 candidate controls (1-7 per CYP450). Sourced neat compound, resolubilized at 50mM and re-testing at 0.2% DMSO

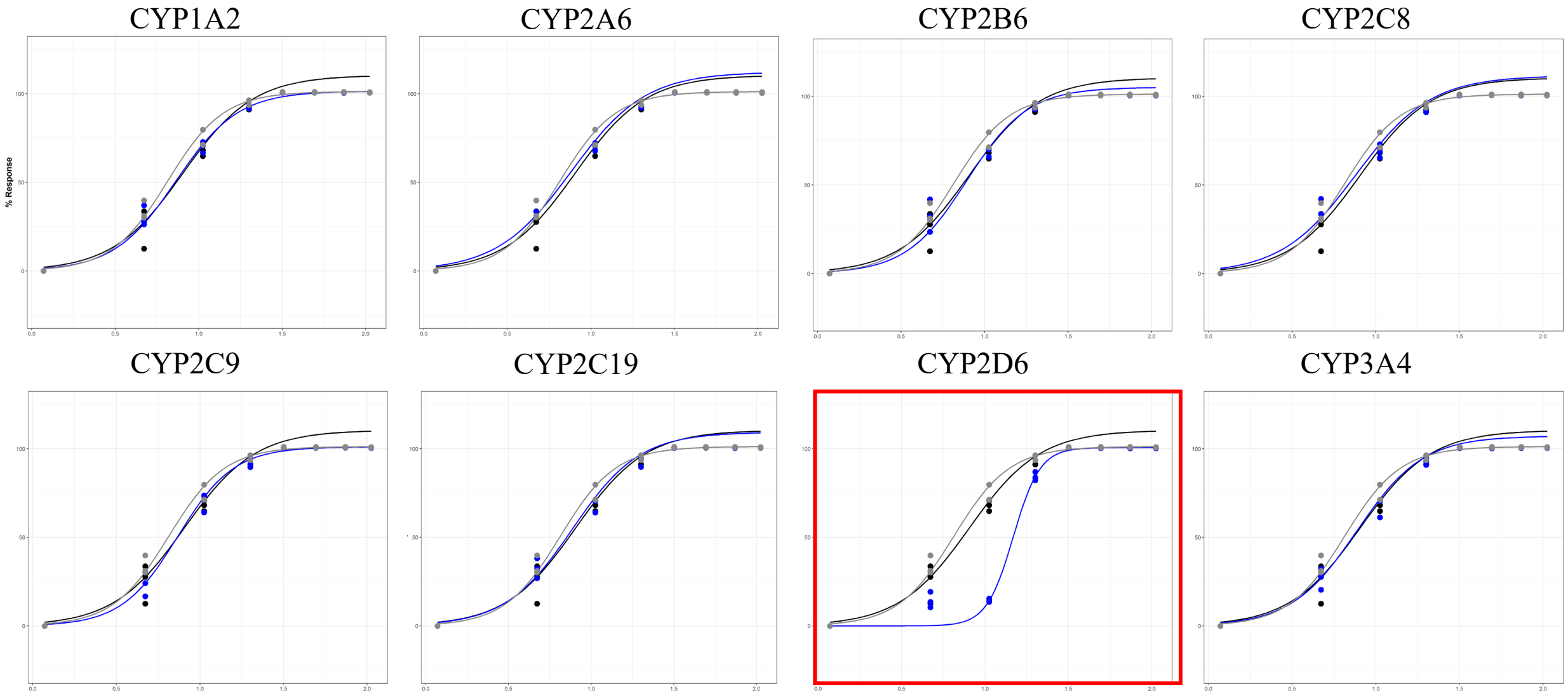
Bioactivation of 8-Hydroxyquinoline by CYP2A6



Bioactivation of 1,4-Diaminoanthraquinone by CYP3A4



Deactivation of Benzalkonium chloride by CYP2D6



Current Status and Future Directions

Androgen Receptor

- **Dec 2021:** Complete selection of CYP450 controls and finalize AR antagonist mode protocol
- **Jan-Jun 2022:** Tiered screening of 1,800-2,200 ToxCast chemicals in AR antagonist mode
- **July-Sept 2022:** Data analysis and manuscript submission
- **Jan 2023-Sept 2025:** Retrofit AR2 assay with AIME (extracellular) and re-screen the same ~2,000 ToxCast chemicals in AR antagonist mode to compare metabolism retrofit strategies tested under identical conditions (proposed for next research plan)

Estrogen Receptor

- **Dec 2021:** Validation of ER-α NanoBit assay with reference agonists and antagonists
- **Jan-Jun 2022:** Identify CYP450 controls and finalize ER-α agonist mode protocol
- **July-Dec 2022:** Screening of 1,800-2,200 ToxCast chemicals in ER-α agonist mode
- **Jan-Dec 2022:** Generate stable ER-β NanoBit cell line, validate, develop protocol

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