Metabolism Retrofit Strategies for ToxCast Assays

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Science and/or Decision Context

The EPA's ToxCast program utilizes a wide variety of high-throughput screening assays (HTS) to assess chemical perturbations of molecular and cellular endpoints. A limitation of many HTS assays used for toxicity assessment is the lack of xenobiotic metabolism (XM) which precludes the detoxification as well as toxic bioactivation of chemicals tested *in vitro*, thereby potentially mischaracterizing the hazard posed by these chemicals. To address this deficiency, we are pursuing two parallel approaches to retrofit HTS assays with XM. The first approach generates metabolites in the assay matrix (buffer/medium) in a cell-free manner and models the effects of circulating metabolites. The second approach uses a mix of human cytochrome P450 (CYP) mRNAs to generate metabolites within test cells and model the direct effect of proximal metabolites.

Approach (with timeline)

Approach #1: Extracellular Protocol

- Fabrication of custom microplate lids **FY16**
- Develop alginate microsphere protocol **FY16**
- Characterization of CYP activities under cell-based and cell-free conditions **FY17**
- Quantitative measure of metabolite formation for key CYP enzymes **FY17**
- Establish coupling protocol for cell-free and cell-based assay **FY17**
- Deploy to selected ToxCast assays **FY17-18**
- Wide-scale deployment to ToxCast assays **FY18**

Approach #2: Intracellular Protocol

- Develop modified mRNA delivery protocol **FY16**
- Synthesize panel of modified human CYP mRNAs **FY16**
- Characterization of multiplexed CYP activities **FY17**
- Quantitative measure of metabolite formation for key CYP enzymes **FY17**
- Determine minimal metabolism requirements for cell types used in ToxCast **FY17**
- Deploy to selected cell-based ToxCast assay **FY17-18**
- Wide-scale deployment to cell-based ToxCast assays **FY18**

Preliminary Results

Approach #1: Extracellular Protocol

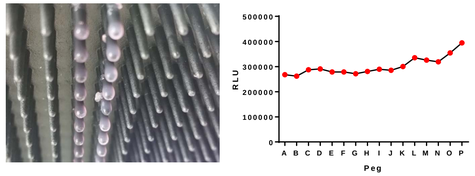

Figure 1: Alginate Immobilization of Metabolic Enzymes (AIME) protocol allows stamping of custom 96- and 384-well lids with microspheres containing human hepatic liver homogenate (left). Well-to-well variation of CYP3A4 activity in a single column of 384-well scale AIME microspheres containing human hepatic liver homogenate.

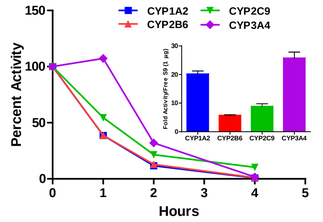

Figure 2: AIME-encapsulated human liver homogenate maintains CYP enzymatic activity over time under cell culture conditions.

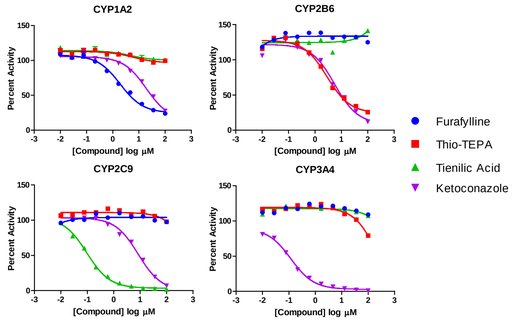

Figure 3: Encapsulated CYP enzymes targeted by known small molecule inhibitors.

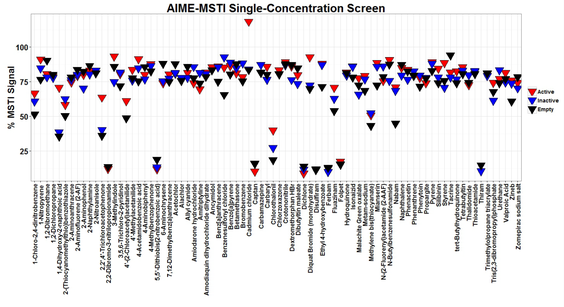

Figure 4: AIME deployment to HTS assay to identify electrophilic metabolites.

Approach #2: Intracellular Protocol

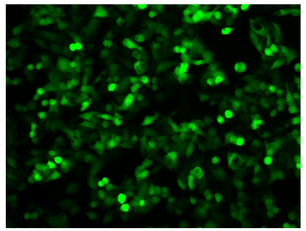

Figure 5: Chemically-modified mRNA encoding enhanced green fluorescent protein (EGFP) used to establish and optimize mRNA delivery protocol in human HepG2 cells.

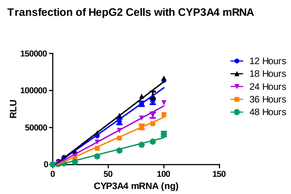

Figure 6: Modified mRNA encoding human CYP3A4 used to identify maximal enzyme activity post-delivery in human HepG2 cells.

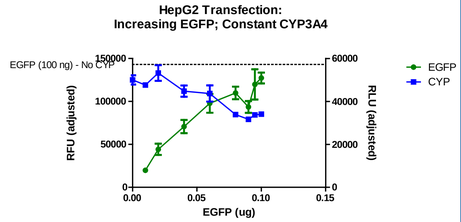

Figure 7: EGFP and CYP3A4 co-delivered to understand how multiplexing genes impacts CYP enzyme activity in human HepG2 cells.

Anticipated Products/Impacts

Products:

- Manuscript detailing AIME protocol using custom lids, characterizing key enzyme activities and formation of known metabolites (FY17)
- Manuscript and data set for ToxCast assay coupled with AIME protocol using active and inactivated human liver homogenate, demonstrating impact of metabolism on assay endpoint (tentatively MSTI assay; FY17)
- Manuscript describing the synthesis of modified mRNA panel of CYP genes and mRNA delivery protocol characterizing key enzyme activities and formation of known metabolites (FY17)
- Manuscript and data set for ToxCast cell-based assay using cells with and without multiplexed CYP mRNAs pooled to mimic human liver (tentatively- BG1/luc ER transactivation assay; FY17)

Impacts:

- The extracellular protocol has the potential to retrofit every ToxCast assay with XM activity
- The extracellular protocol can be expanded beyond human liver homogenate to include homogenates from other tissues of recombinant sources of enzymes
- The intracellular protocol can only retrofit cell-based ToxCast assays, but can model the direct effects of reactive metabolites and with a user-defined gene mix
- Collectively, these two approaches seek to improve the data quality from HTS assays by expanding the chemical test space from parent compounds to include the constellation of resulting metabolites

References

1. Lee et al. (2013) Sens. Actuators, B 177: 78-85
2. McCallum et al. (2013) J. Biomol. Screening, 18(6): 705-713
3. Warren et al. (2010) Cell Stem Cell, 7(5): 618-630