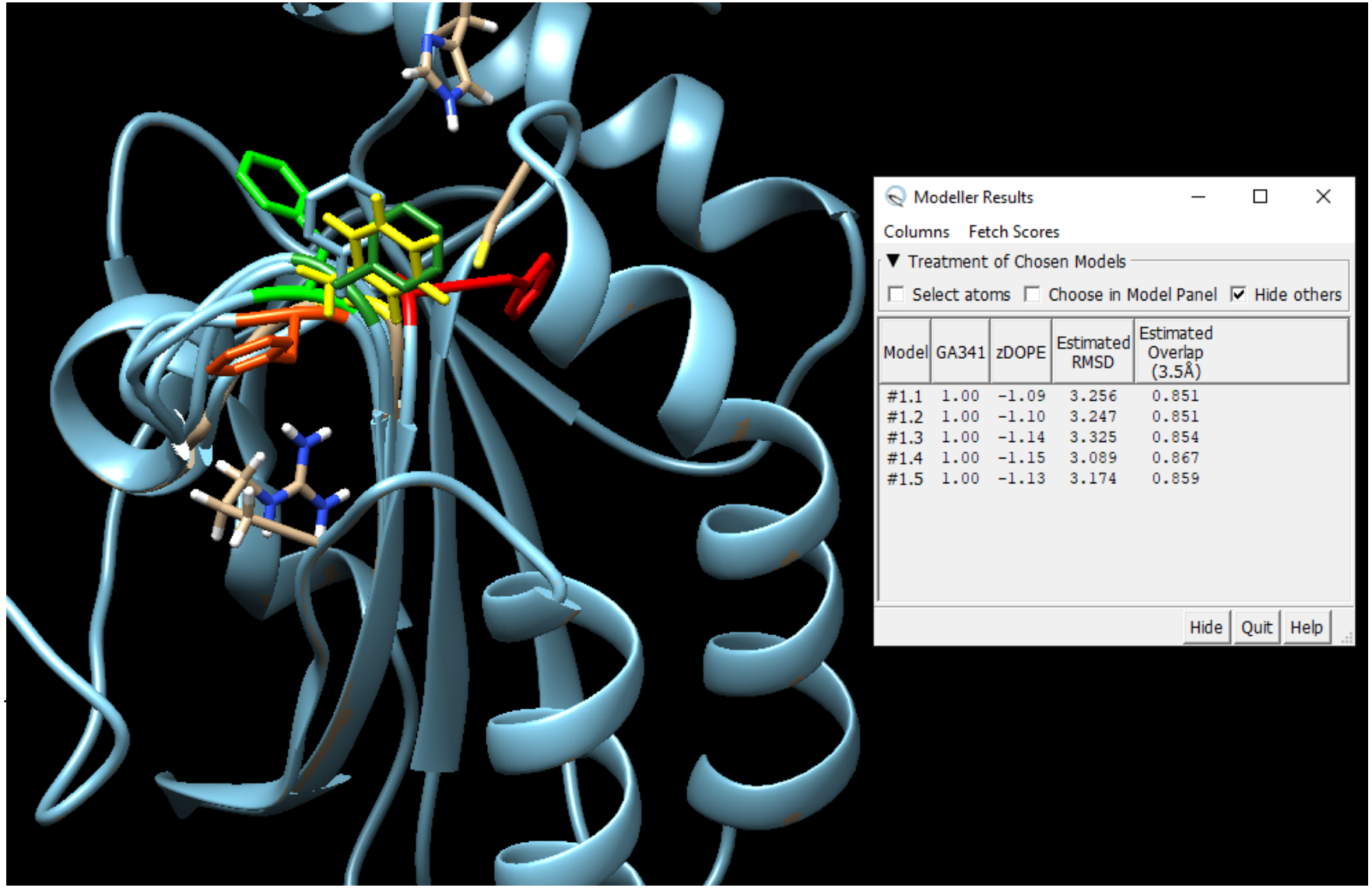
Human DIO3 cross-species-based site-directed mutagenesis modeling & docking

US EPA Great Lakes Toxicology and Ecology Division (GLTED) Bioinformatics Team

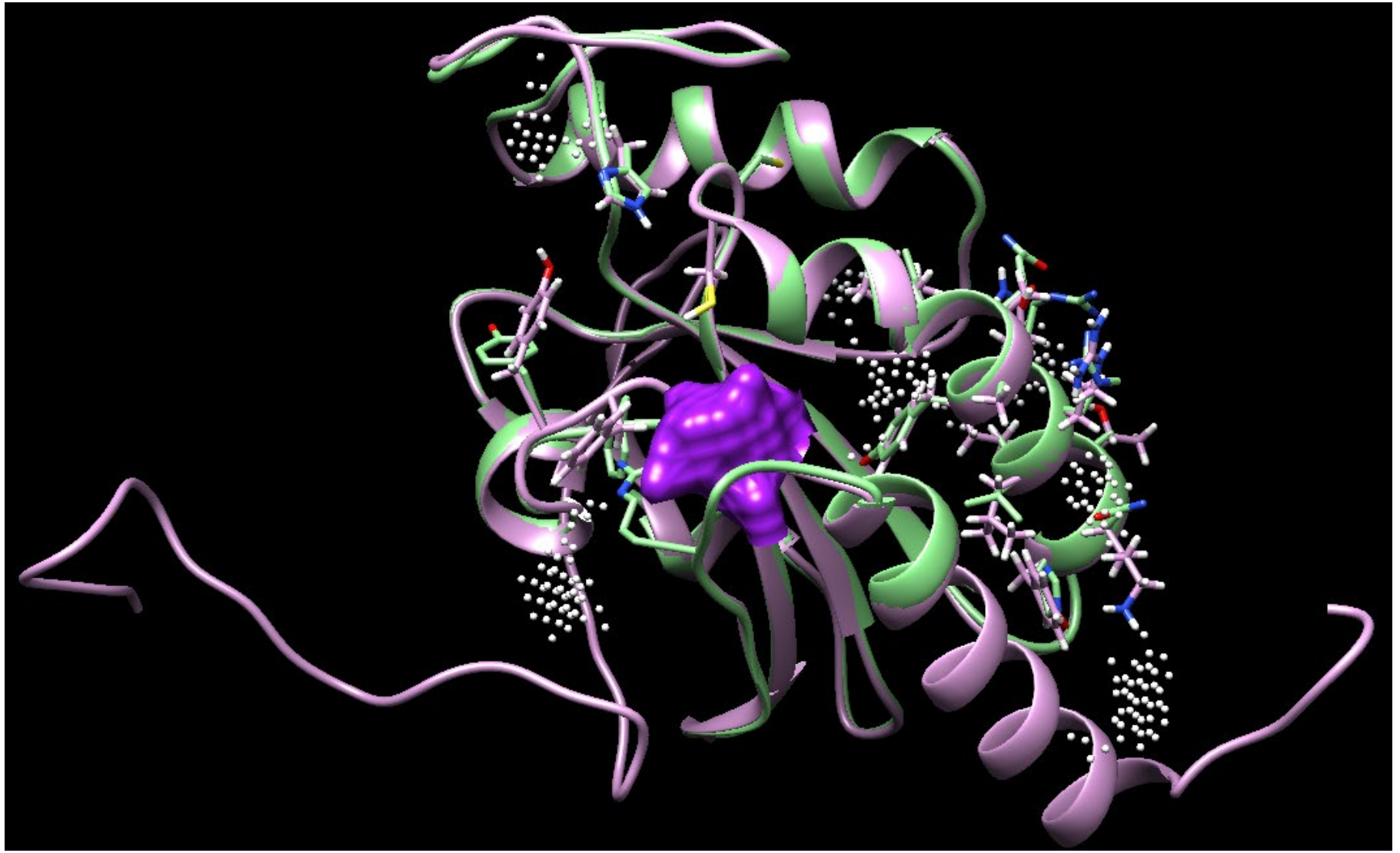
- **Purpose:** *Empirical in vitro chemical screening across the human type 3 iodothyronine deiodinase (hDIO3) mutants will provide a hierarchy of chemical binding affinity based on IC50.*
- *This data will be compared with results of virtual docking of the same set of chemicals to molecular models of the hDIO3 wildtype and in silico mutated protein sequences to test the accuracy of virtual docking pipeline strategies.*
- *The comparison is also intended to provide a proof-of-concept demonstrating validity of modeling and virtual chemical screening of protein homologs across species.*

Human DIO3 cross-species-based site-directed mutagenesis modeling & docking

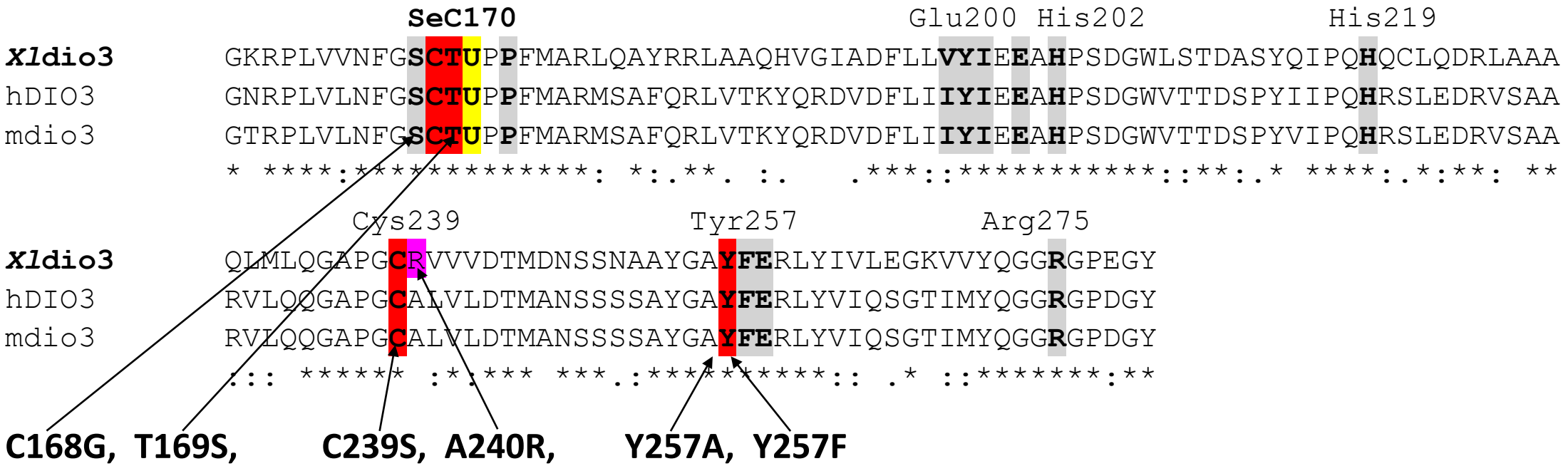
- I-TASSER→Chimera with MODELLER to remodel loops



- POCASA and FTMap to identify pockets and cavities
- Chimera/Autodock Vina



Selected mutations and cross-species dio3 sequence comparison

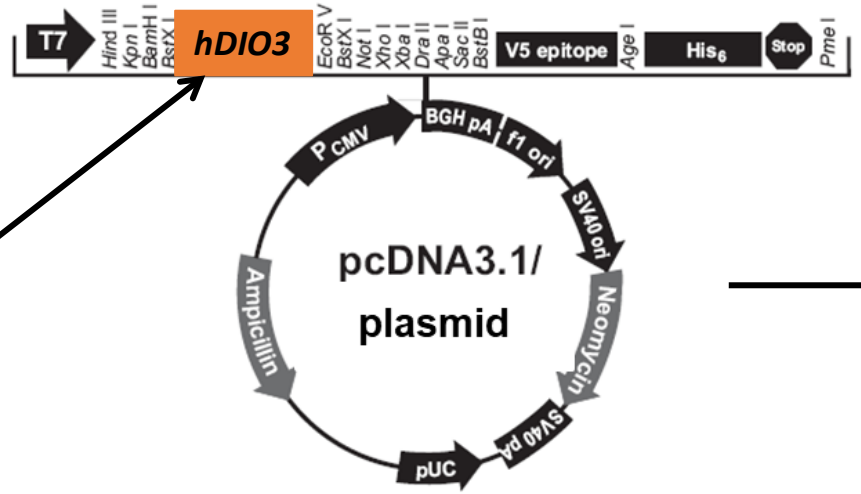


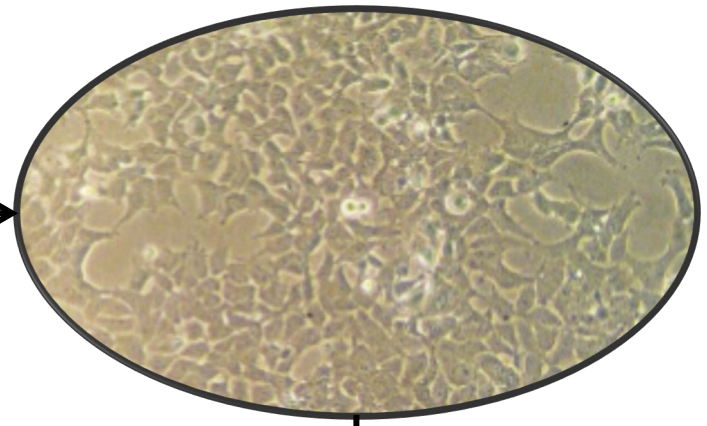
Plasmids with hDIO3 gene inserts synthesized by GenScript each have a single mutation.

Test mutants using deiodinase *in vitro* screening assay

Transfect each SDM mutant plasmid construct into HEK293 cells.

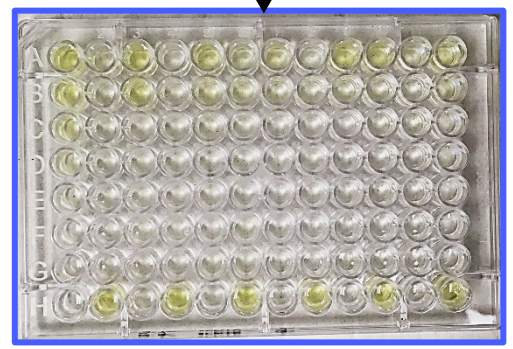
hDIO3 mutants:
C168G
T169S
C239S
A240R
Y257A
Y257F

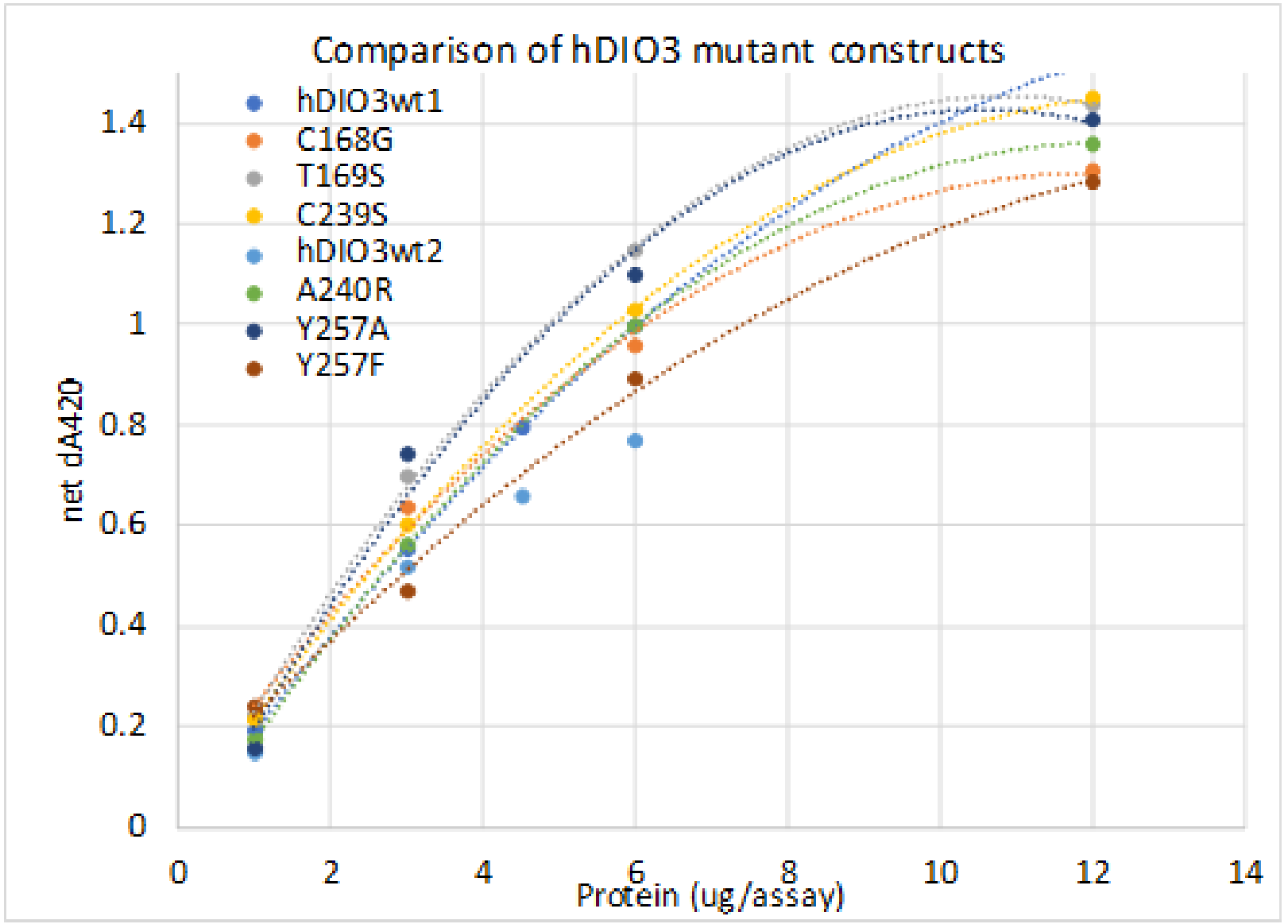




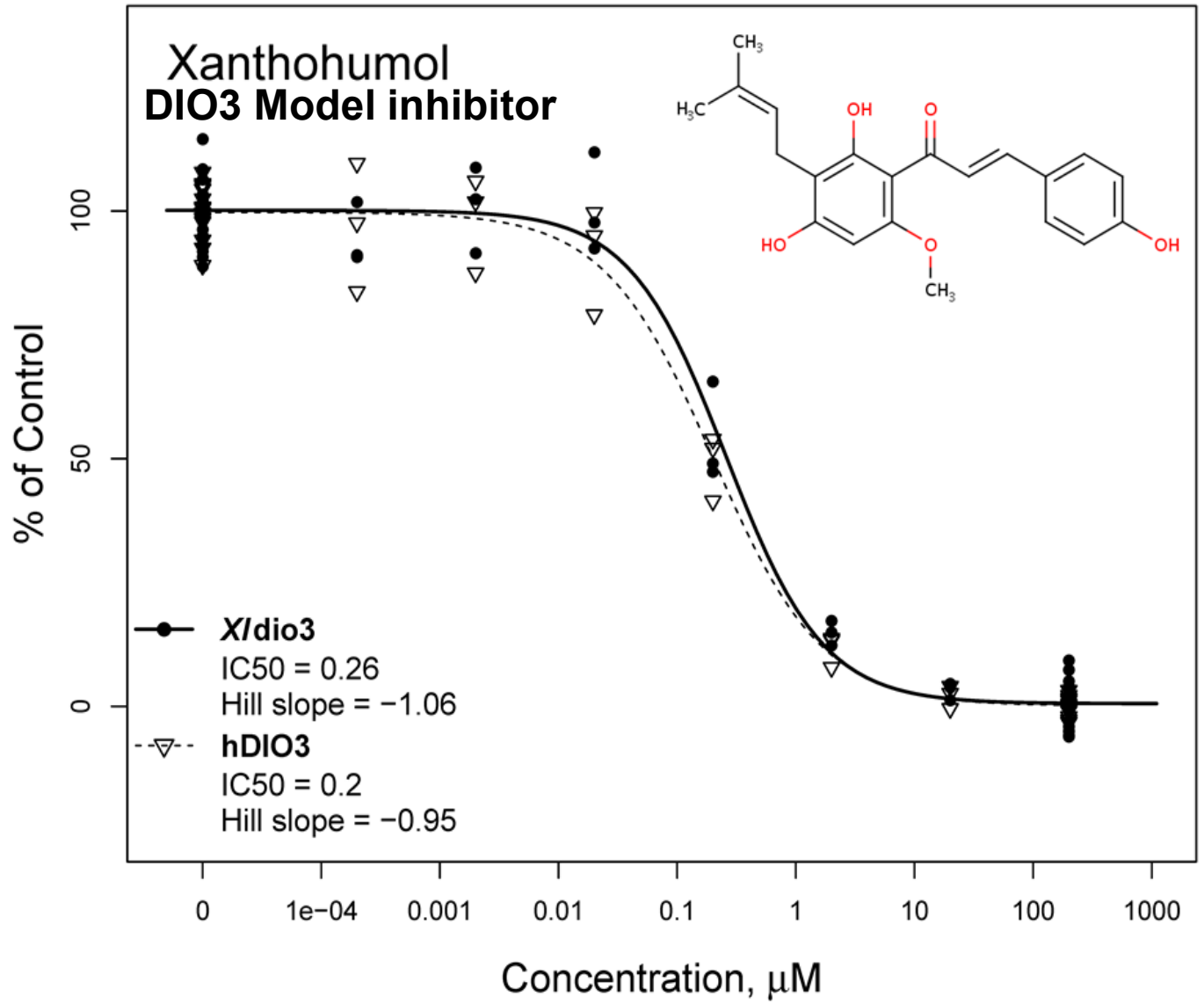
Lyse/sonicate cells. Incubate 3h with T3 substrate + DTT cofactor in HEPES buffer. **Adjust protein content for activity within optimal dynamic range.**

Sandell-Kolthoff assay
detect free iodide at absorbance of 420 nm in a 96-well plate reader.

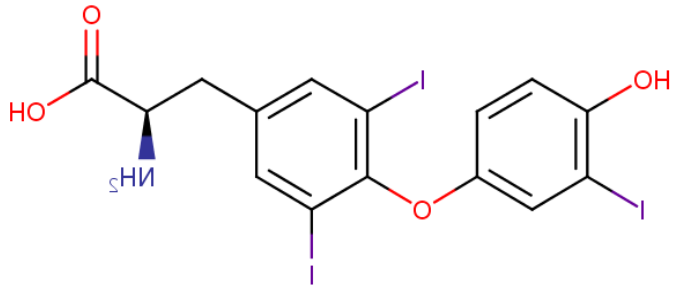



Comparison of hDIO3 mutant constructs
hDIO3wt1
C168G
T169S
C239S
hDIO3wt2
A240R
Y257A
Y257F
net dA420
Protein (ug/assay)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
2
4
6
8
10
12
14

SDM chemicals to be selected from previous in-vitro hDIO3 and Xenopus dio3 screening of environmental chemicals

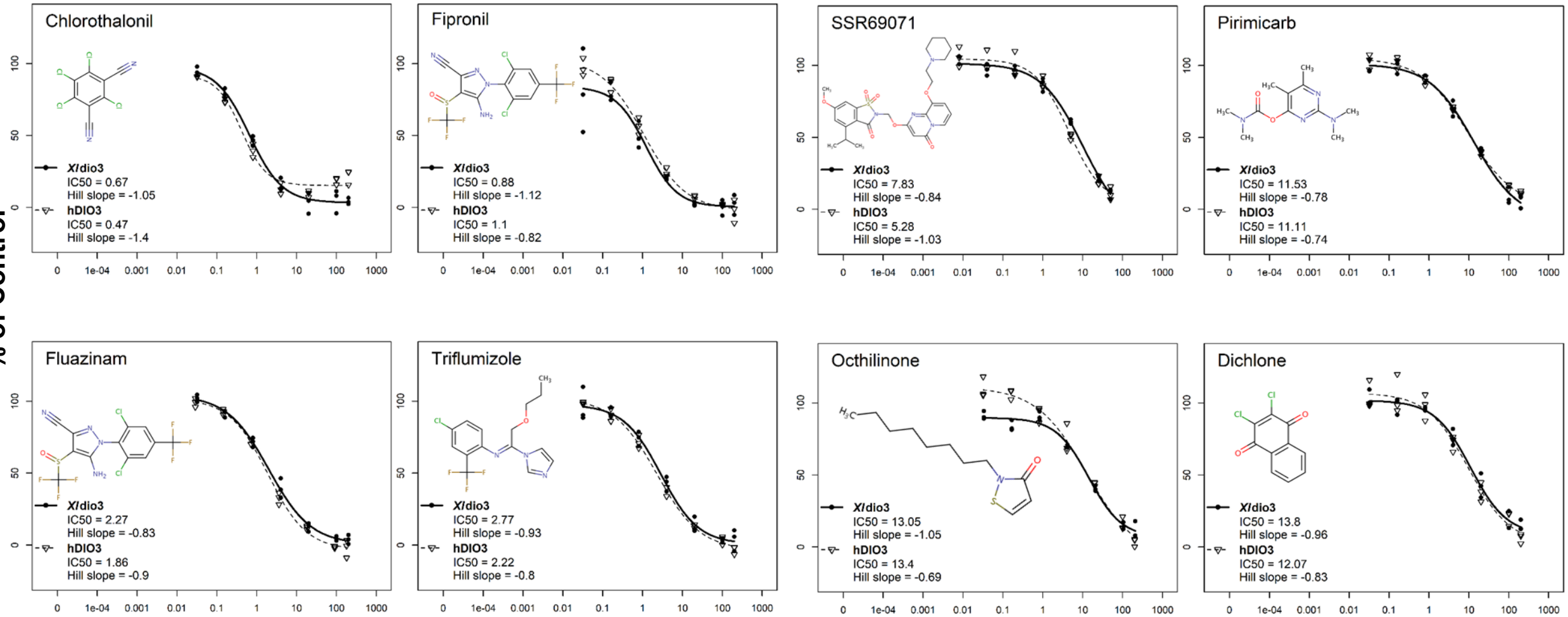


Thyroid hormone (T3) substrate



Due to resources and time, first cut chemicals to be screened will be limited to eleven plus xanthohumol (fits in one 96-well plate; triplicate = 3 plates x 7 constructs) x 2 if we also run T4 substrate

Potential specific, competitive inhibitors of human and Xenopus type 3 deiodinase



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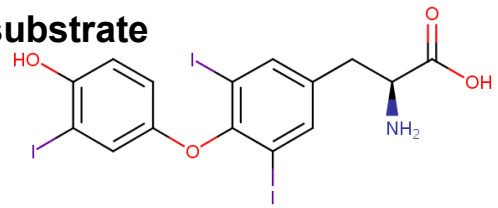
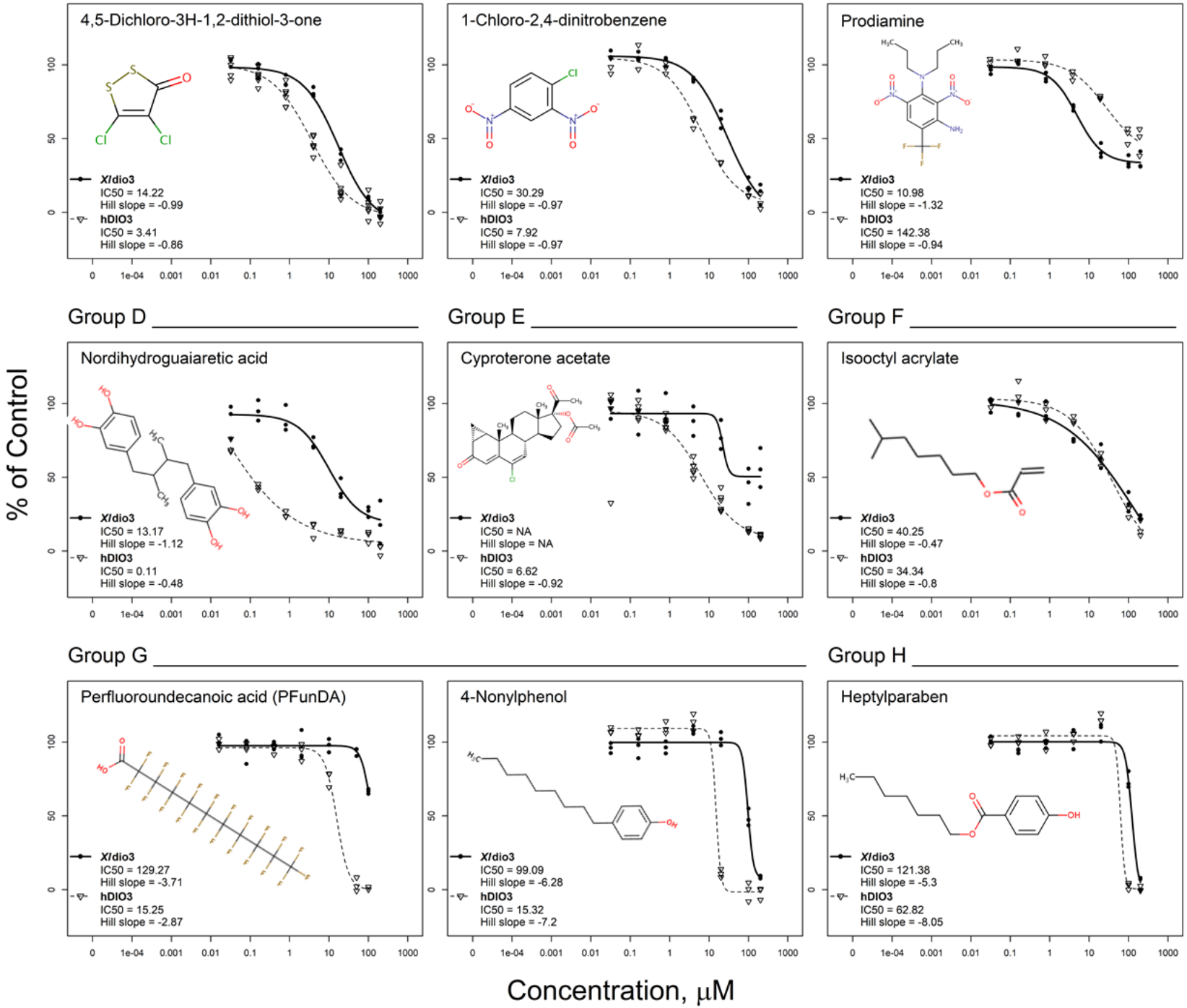


Example concentration-response curves with potential artifacts due to aggregates, micelles or precipitation in the assay, indicated by curve shape:

- steep slope
- incomplete inhibition

This gives us a basis for eliminating chemicals that are likely causing artifacts from consideration for the mutant *in vitro* screening.



Will virtual screening help distinguish between differences in IC50 due to structure vs assay artifact?