

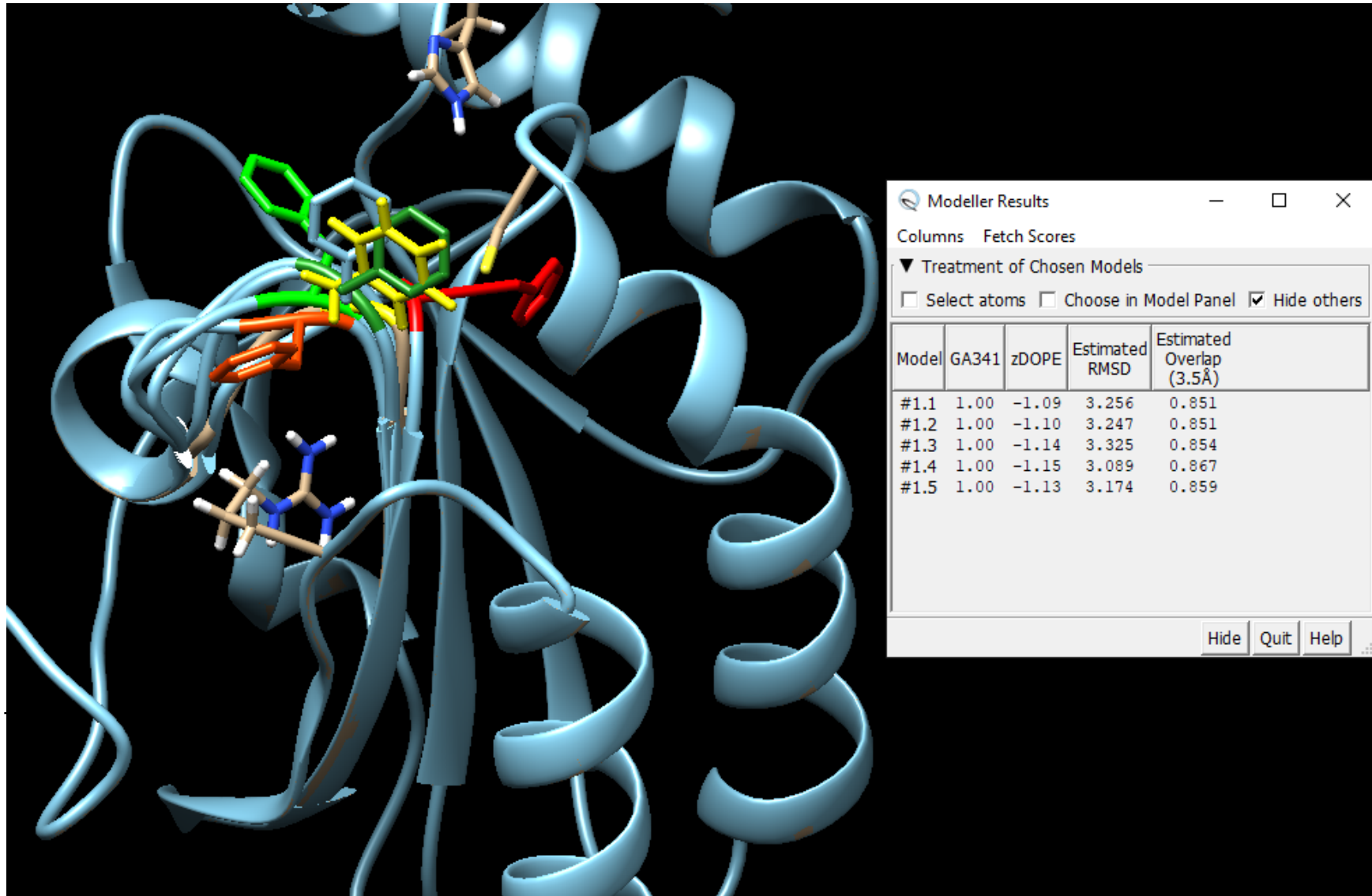
# Human DIO3 cross-species-based site-directed mutagenesis modeling & docking

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

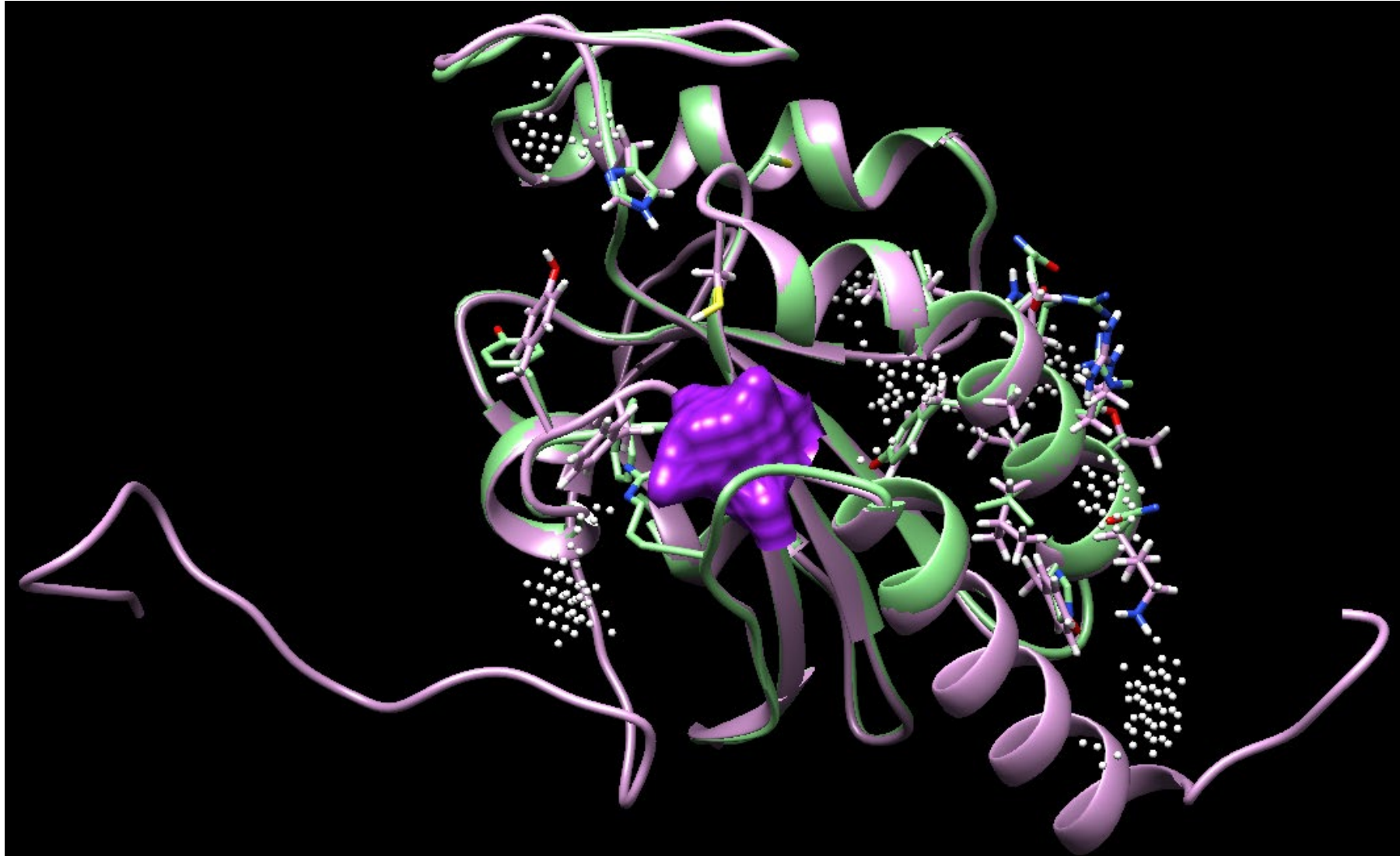
- **Purpose:** *Empirical in vitro chemical screening across the human type 3 iodothyronine deiodinase (hDIO3) mutants will provide a hierarchy of chemical binding affinity based on IC50.*
- *This data will be compared with results of virtual docking of the same set of chemicals to molecular models of 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

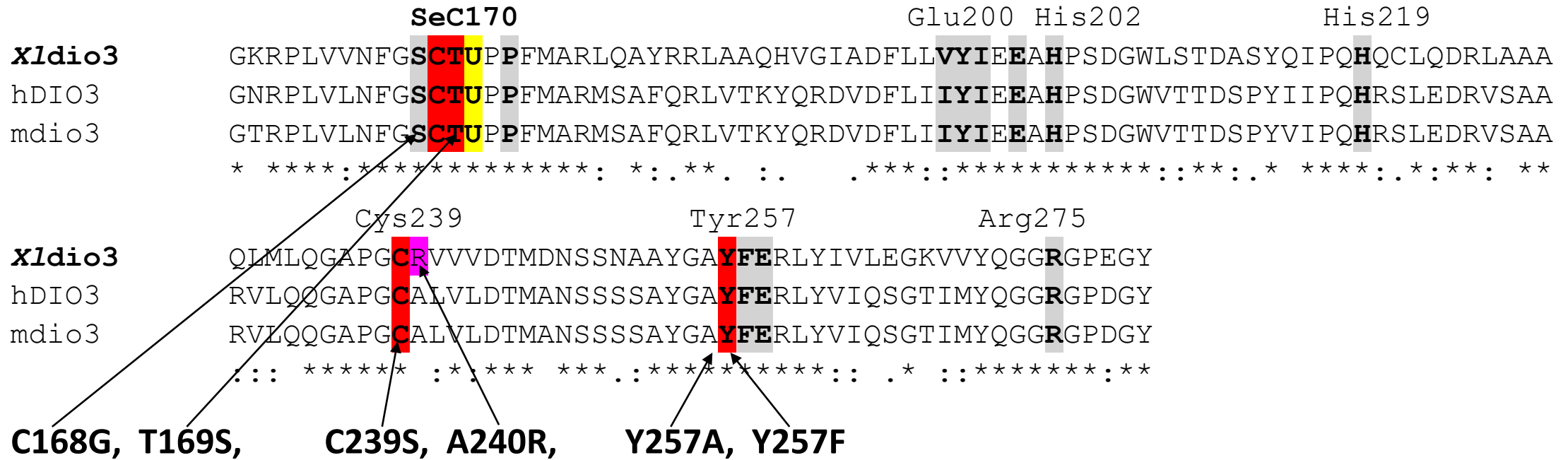
- I-TASSER→Chimera with MODELLER to remodel loops



- 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

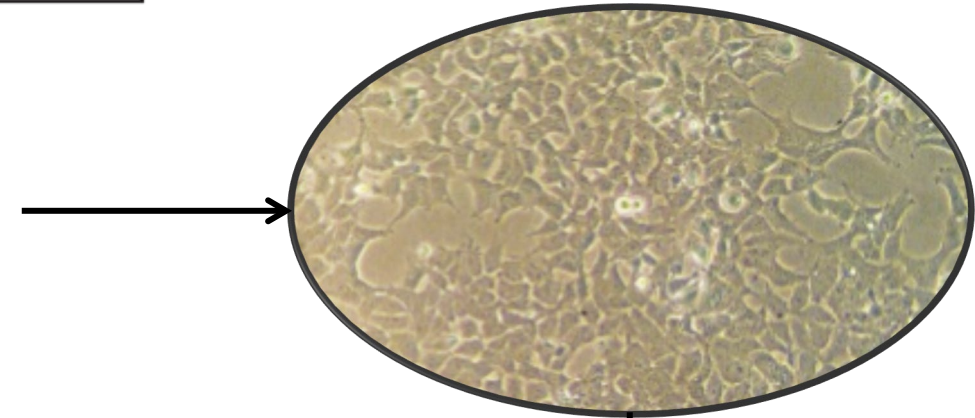
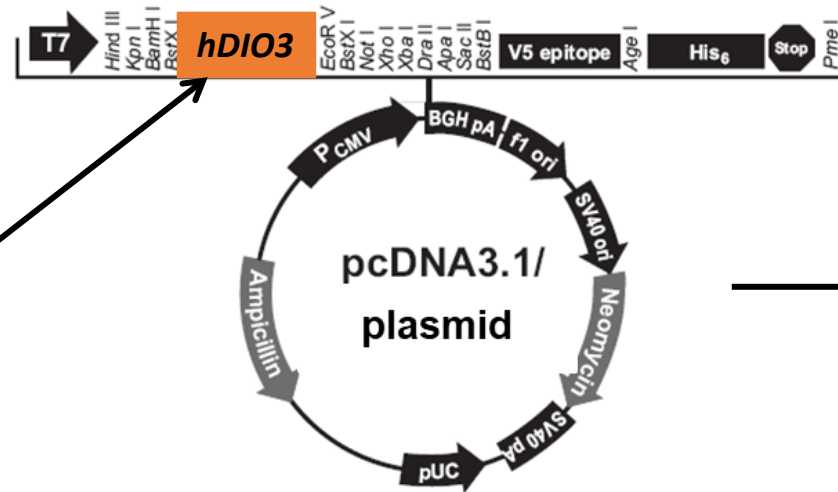
T169S

C239S

A240R

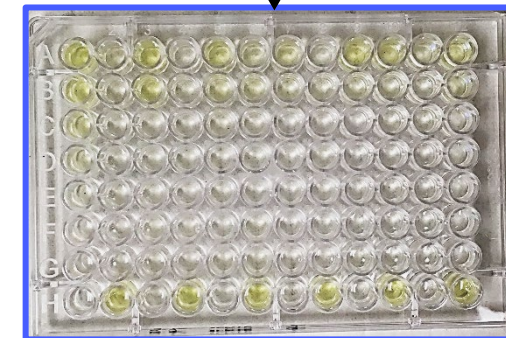
Y257A

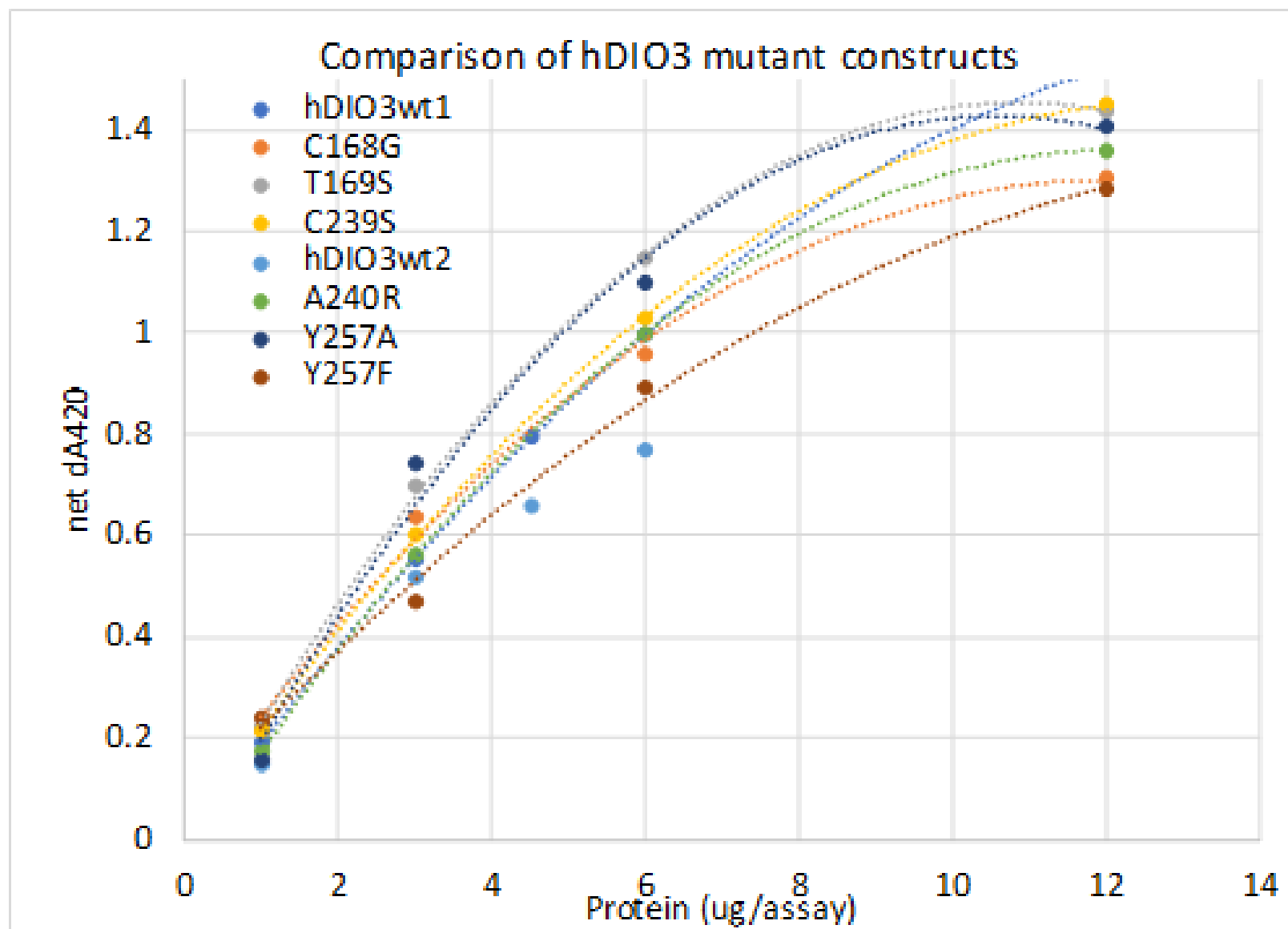
Y257F



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

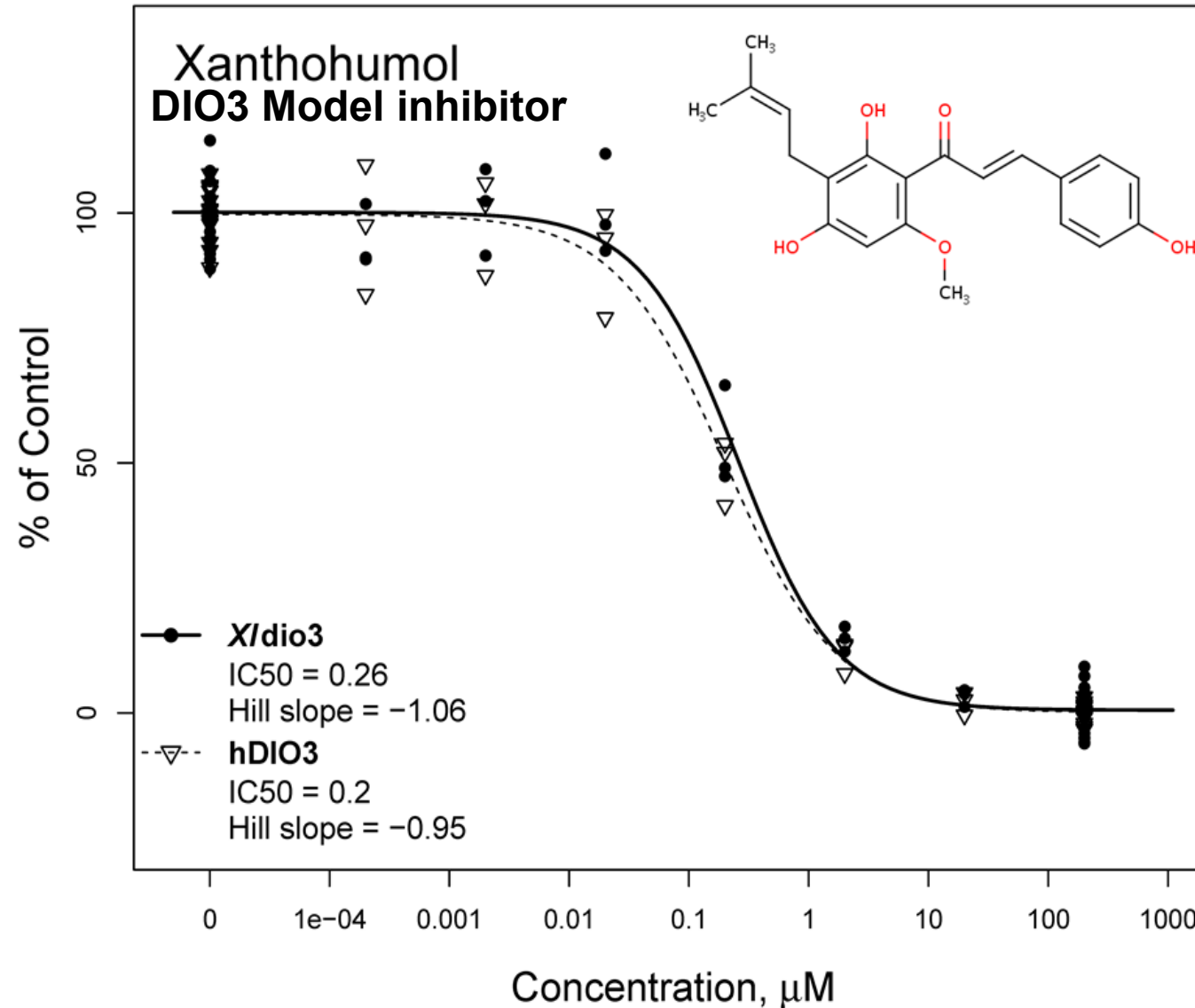
**Sandell-Kolthoff assay**  
detect free iodide at absorbance of 420  
nm in a 96-well plate reader.



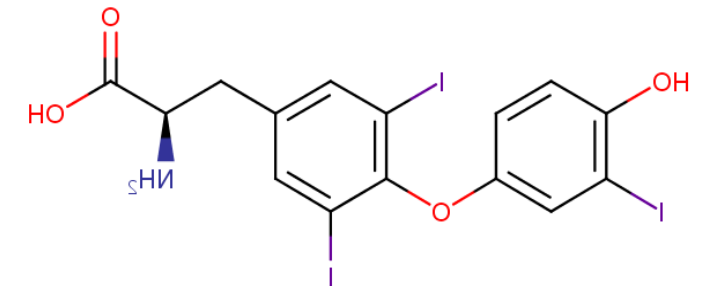




# SDM chemicals to be selected from previous in-vitro hDIO3 and Xenopus dio3 screening of environmental chemicals



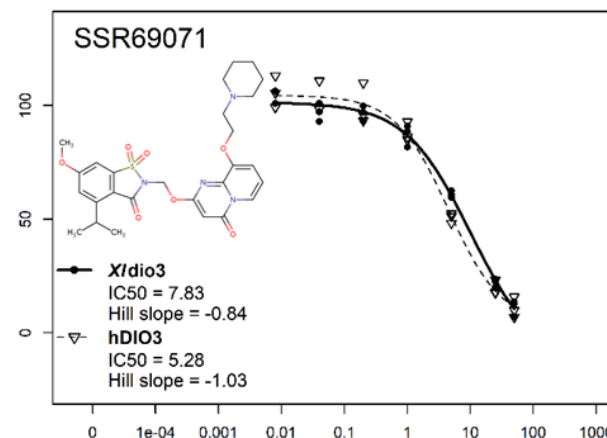
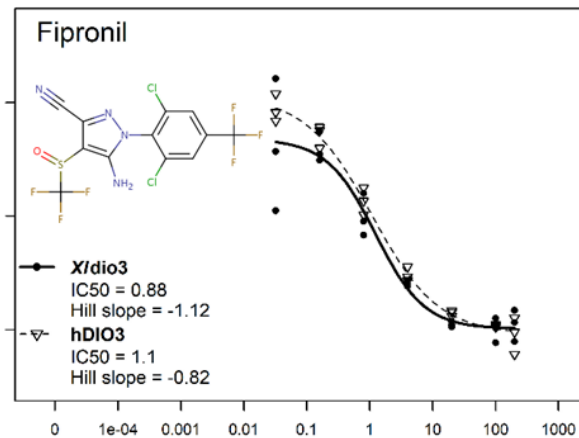
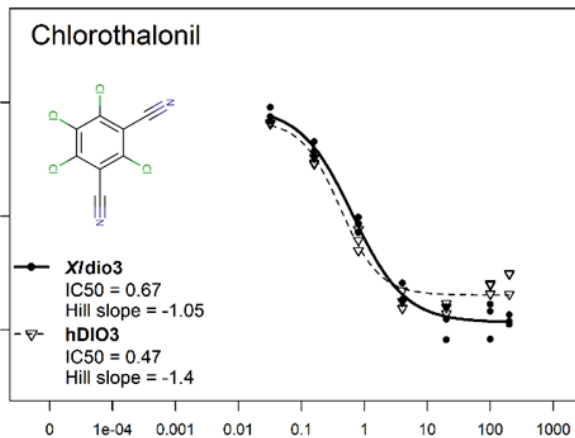
## Thyroid hormone (T3) substrate



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

% of Control

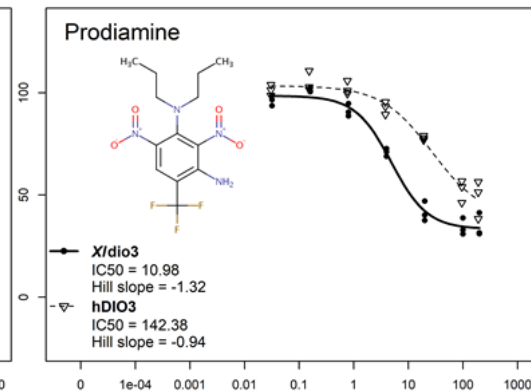
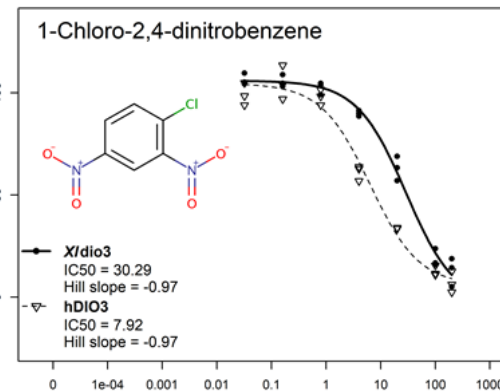
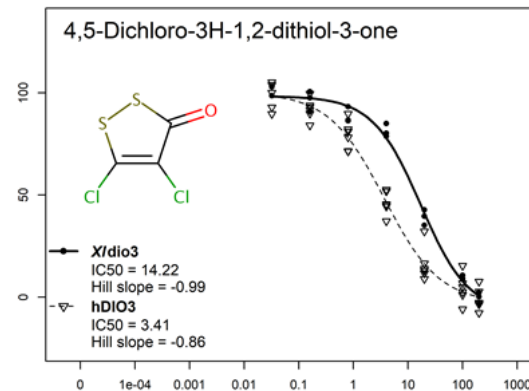




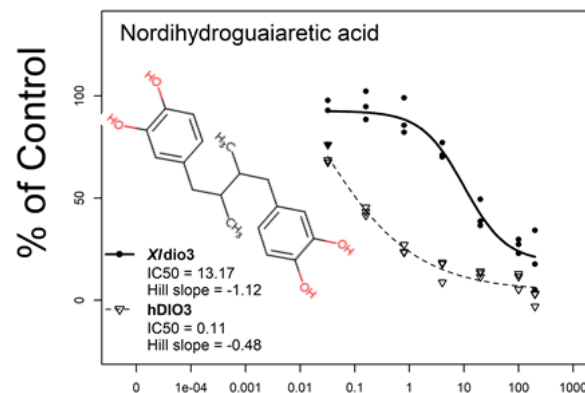
Example concentration-response curves with potential artifacts due to aggregates, micelles or precipitation in the assay, indicated by curve shape:

- steep slope
- incomplete inhibition

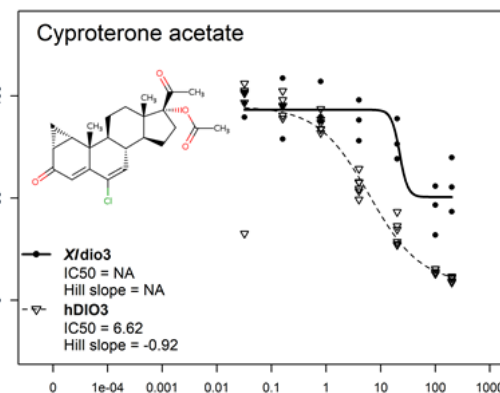
This gives us a basis for eliminating chemicals that are likely causing artifacts from consideration for the mutant *in vitro* screening.



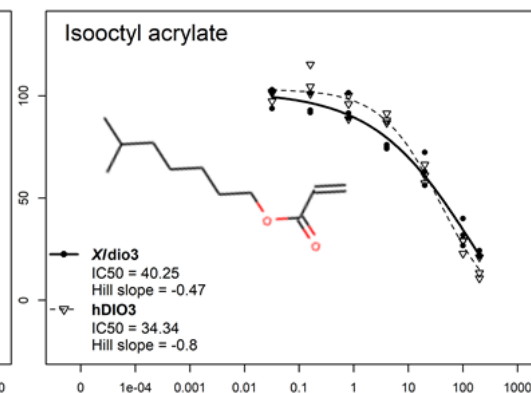
Group D



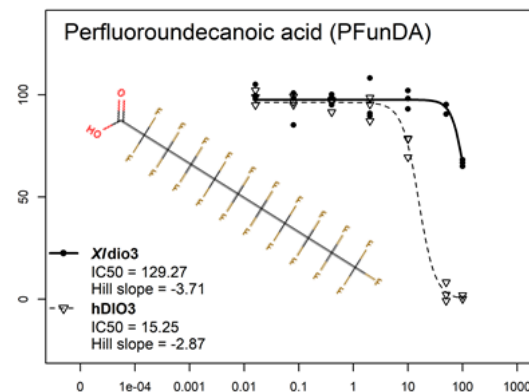
Group E



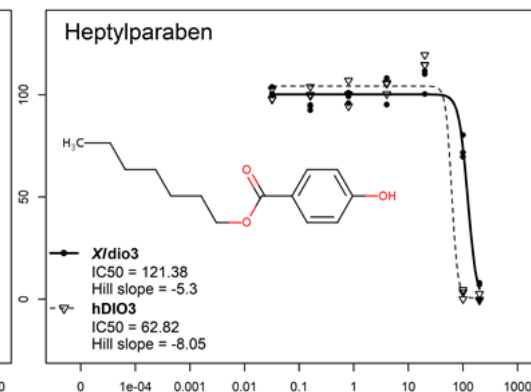
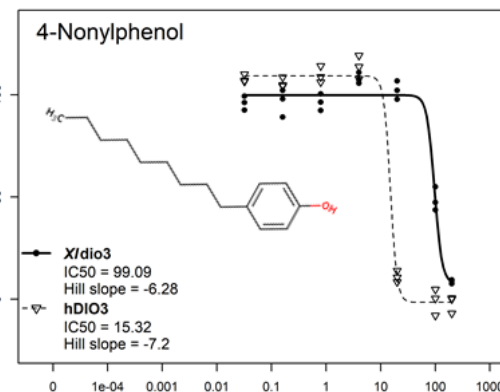
Group F



Group G



Group H



Concentration,  $\mu\text{M}$

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

