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Bioinformatics for Cross-Species Chemical Susceptibility Prediction and Interpretation of In Vitro Screening Results: Case Study using a Thyroid Deiodinase Enzyme

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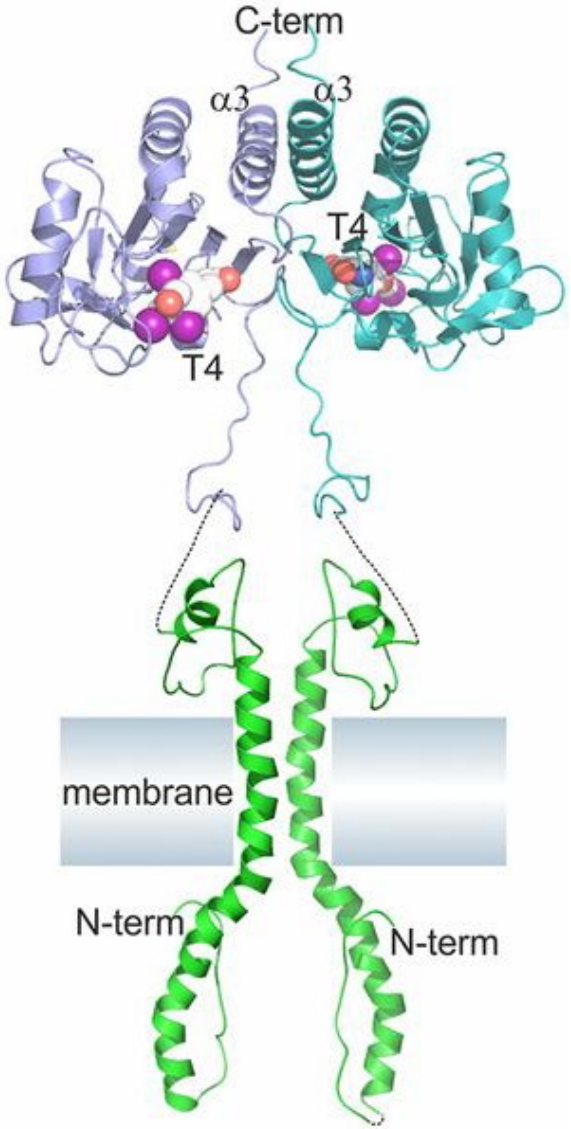
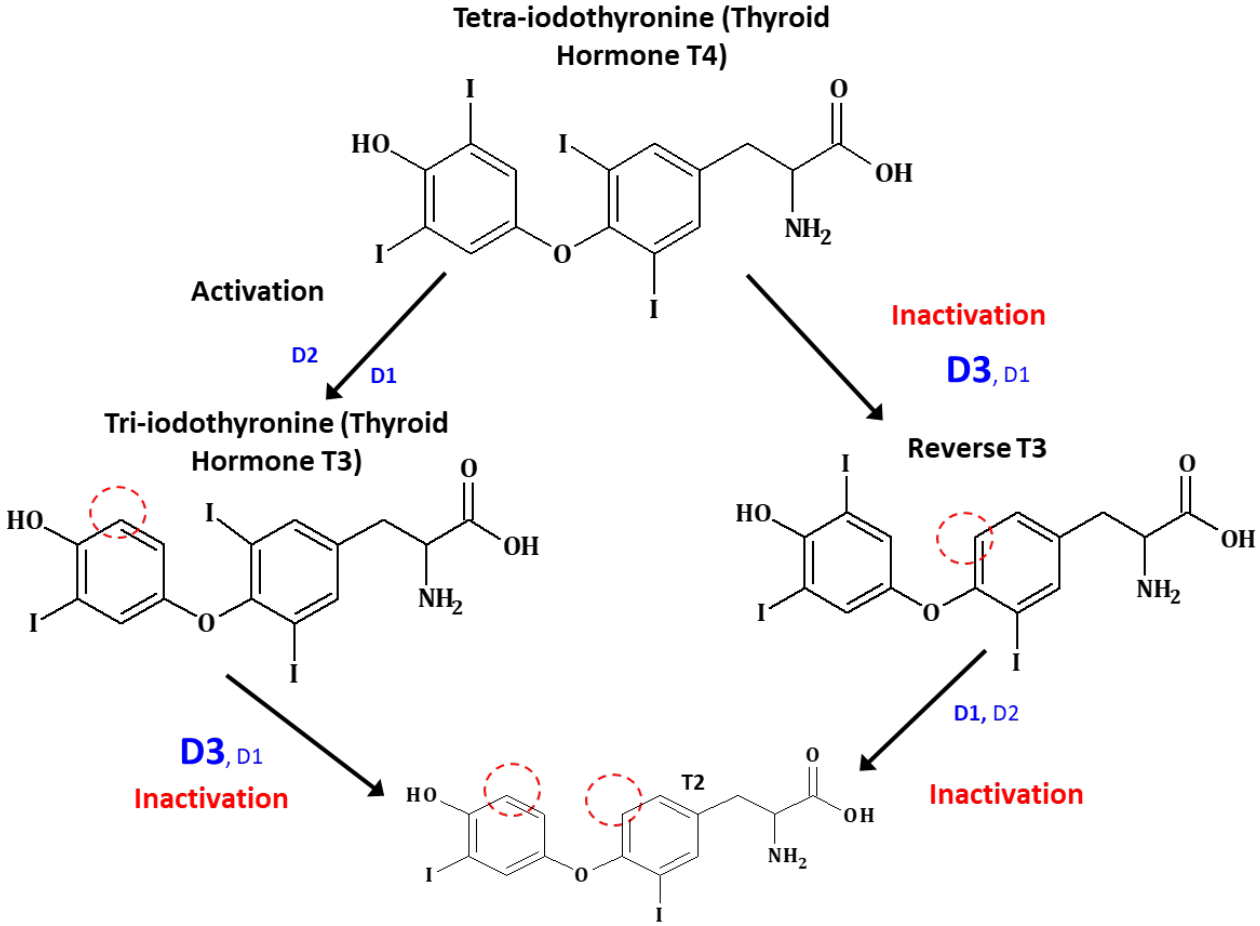
Deiodination of thyroid hormone substrates

Deiodinase enzymes are critical for tissue-specific and temporal control of activation or inactivation of thyroid hormones during vertebrate development.



Dio3 homodimer model. Ulrich Schweizer et al. PNAS 2014;111:29:10526-10531



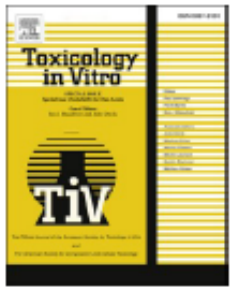
Previous screening

Toxicology in Vitro 73 (2021) 105141

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



Check for updates

Xenopus laevis and human type 3 iodothyronine deiodinase enzyme cross-species sensitivity to inhibition by ToxCast chemicals

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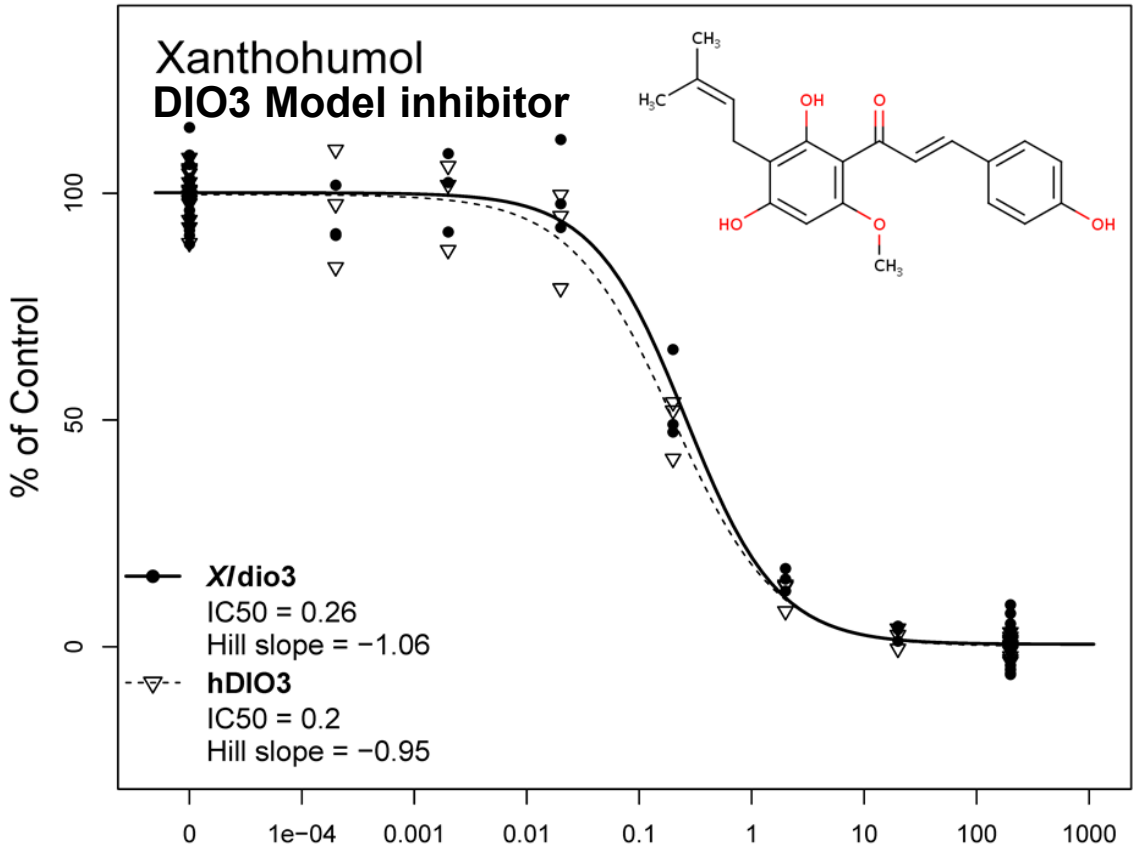
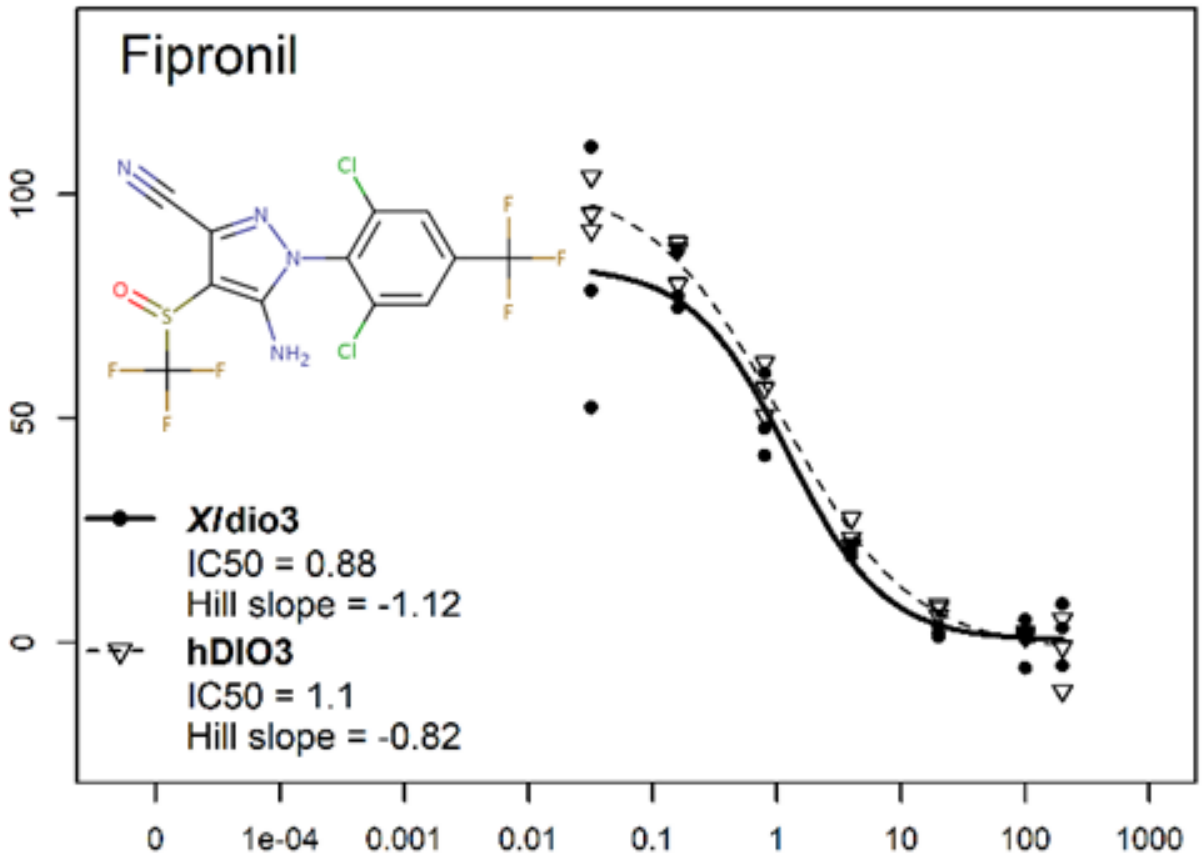
[d] Oak Ridge Associated Universities, Oak Ridge, TN, USA

Previous screening results

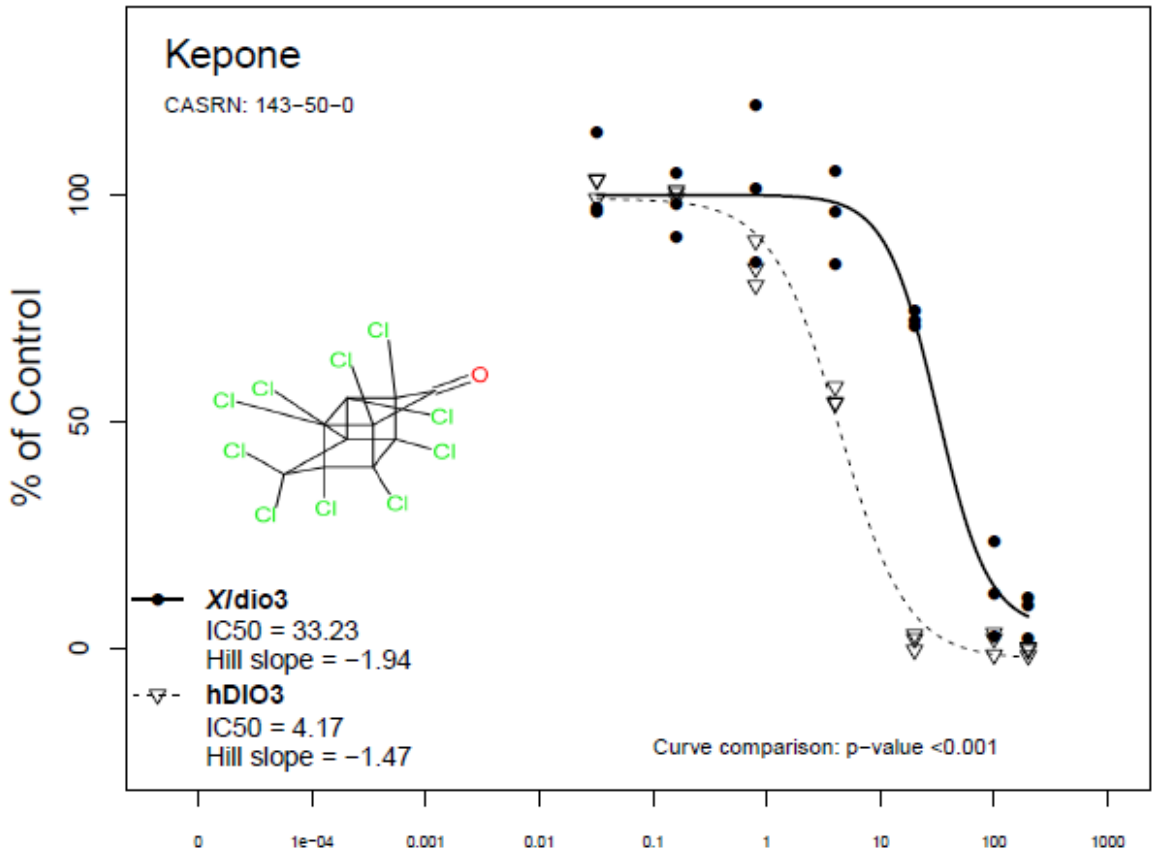
Potent inhibitors with Hill slopes and IC50 **not significantly different** between species

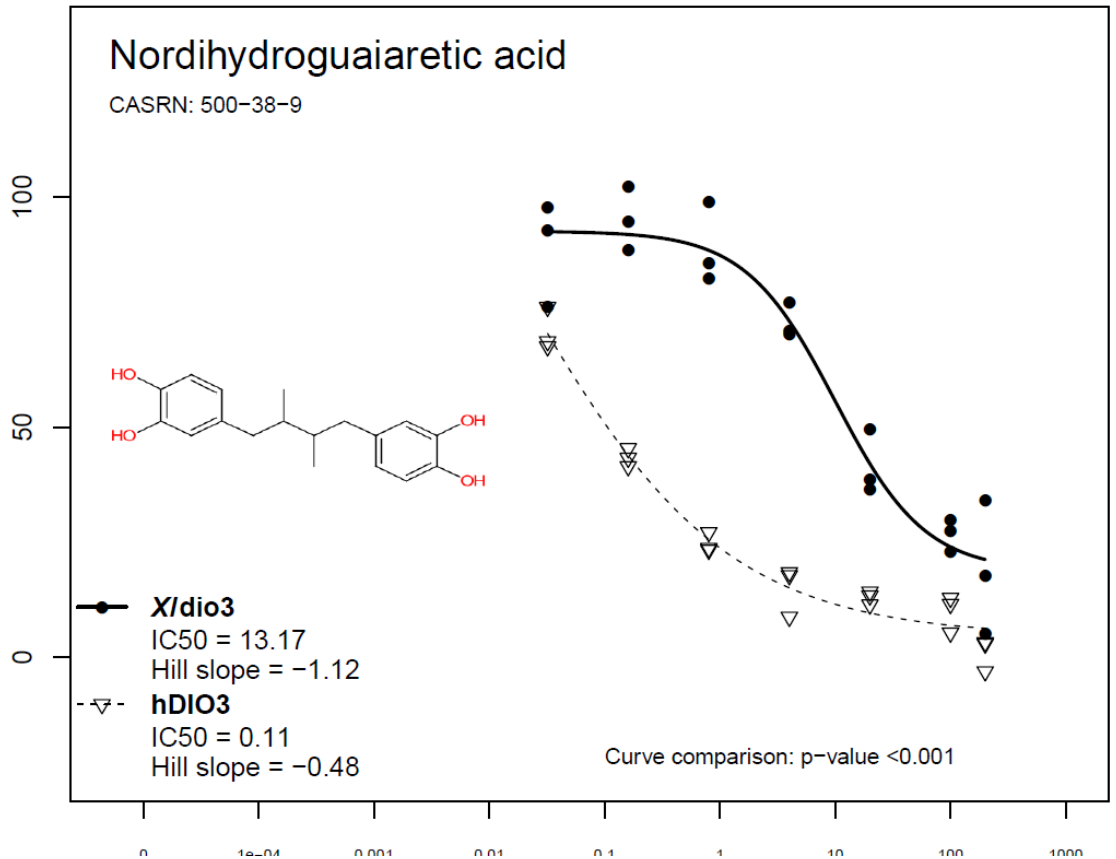




Previous screening results

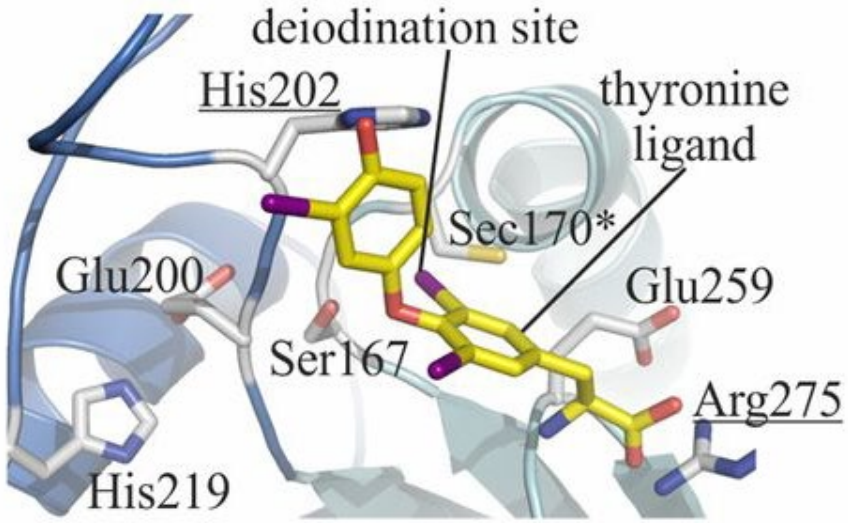
Chemicals for which concentration-response **curves differed** between species potentially due to non-competitive (allosteric) inhibition

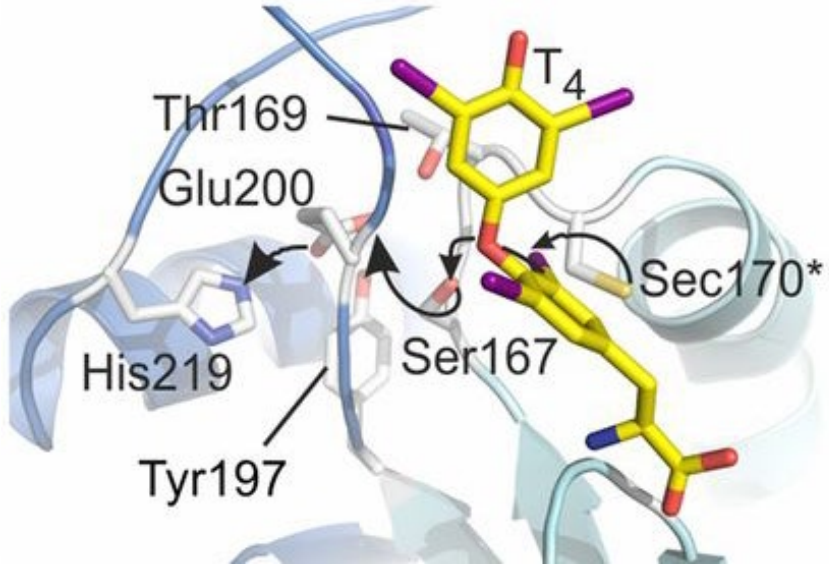


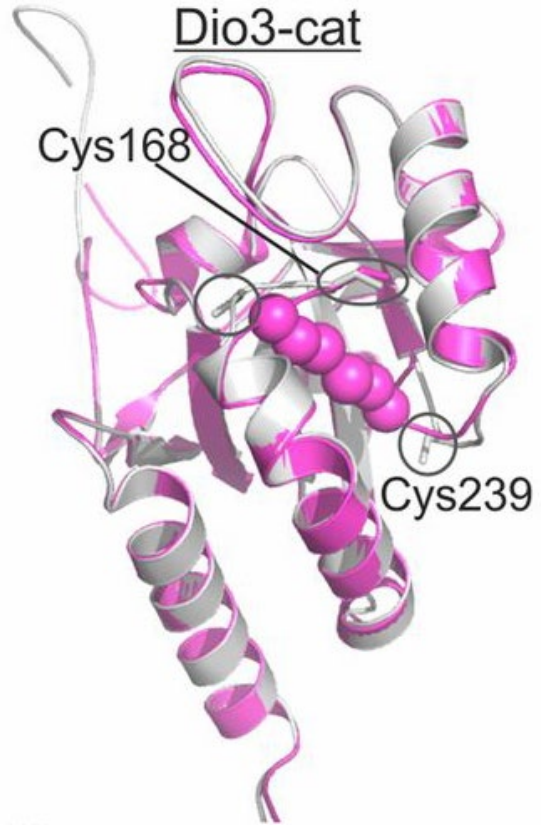


Concentration (μM)

Schweizer et al (2014) determined important catalytic site amino acid residues using mouse dio3





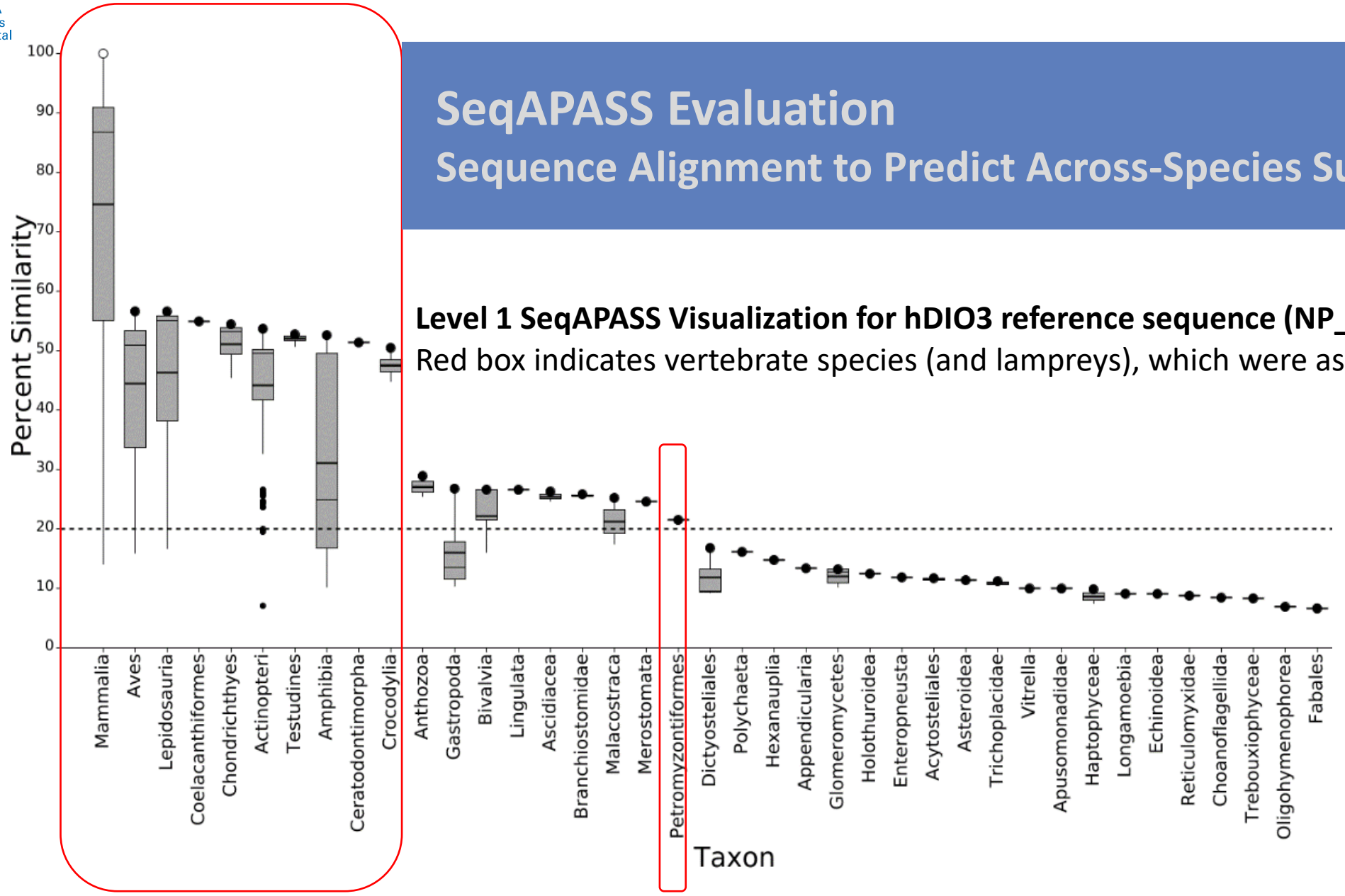


Dio3 structure suggests a catalytic hydrogen bond network and a Prx-like (thiol) reduction cycle.

Ulrich Schweizer et al. PNAS 2014;111:29:10526-10531

SeqAPASS Evaluation

Sequence Alignment to Predict Across-Species Susceptibility



Level 1 SeqAPASS Visualization for hDIO3 reference sequence (NP_001353.4)

Red box indicates vertebrate species (and lampreys), which were assessed in level 3

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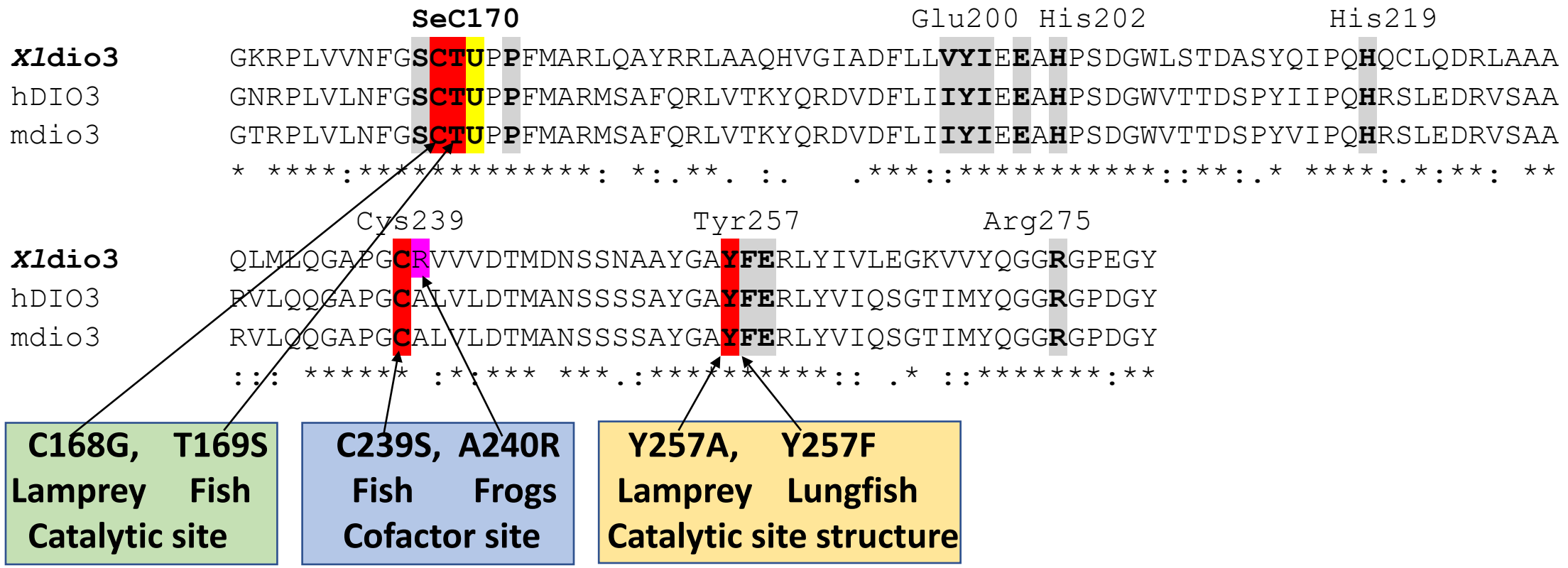
SeqAPASS V6.0 Level 3 Evaluation Primary Results

Amino acid residue alignment with hDIO3. Other critical residue positions were highly conserved.

Total Match | Partial Match | Not a Match | Susceptible Yes | Susceptible No

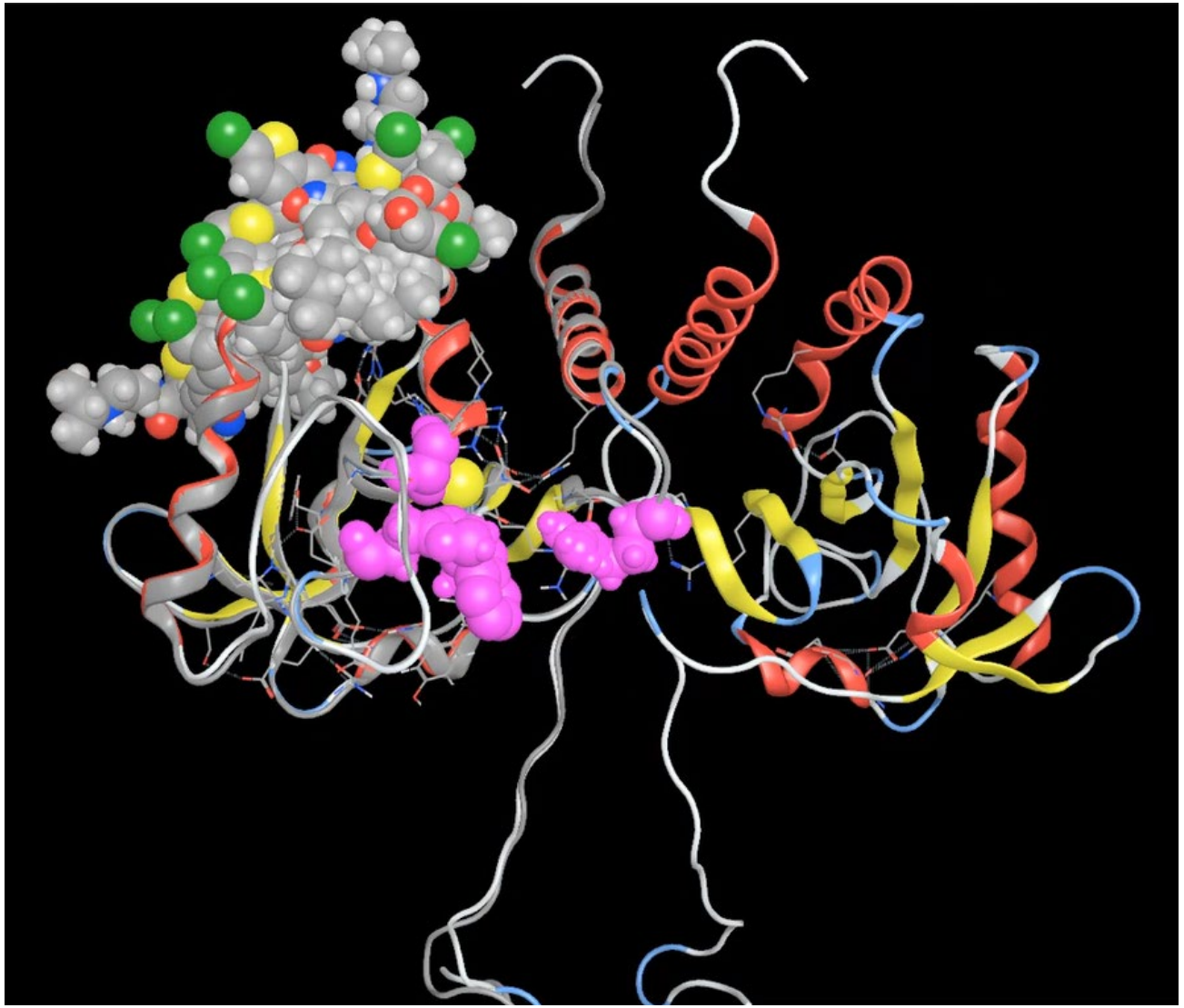
			C168	T169	C239	A240	Y257
Scientific Name	Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5
Homo sapiens	Human	Y	168C	169T	239C	240A	257Y
Latimeria chalumnae	Coelacanth	N	131C	132T	202C	203F	220Y
Clupea harengus	Atlantic herring	Y	122C	123T	193C	194V	211Y
Gadus morhua	Atlantic cod	Y	184C	185T	255C	256A	273Y
Carassius auratus	Goldfish	Y	135C	136S	206S	207A	224Y
Amphiprion ocellaris	Clown anemonefish	Y	123C	124T	194C	195L	212Y
Sciaenops ocellatus	Red drum	N	130C	131S	201S	202N	219Y
Oreochromis aureus	Blue tilapia	Y	123C	124T	194C	195L	212Y
Maylandia zebra	Zebra mbuna	N	130C	131S	201T	202N	219Y
Solea senegalensis	Senegalese sole	N	130C	131S	201G	202N	219Y
Cottoperca gobio	Thornfishes	Y	123C	124T	194C	195P	212Y
Gymnodraco acuticeps	Antarctic dragonfishes	Y	123C	124T	194C	195P	212Y
Morone saxatilis	Striped sea-bass	N	123C	124T	194C	195M	212Y
Micropterus salmoides	Largemouth bass	Y	123C	124T	194C	195L	212Y
Neoceratodus forsteri	Australian lungfish	Y	133C	134T	204C	205L	222F
Eleutherodactylus coqui	Puerto Rican coqui	N	130C	131T	201C	202R	219Y
Xenopus tropicalis	Tropical clawed frog	N	130C	131T	201C	202R	219Y
Xenopus laevis	African clawed frog	N	128C	129T	199C	200R	217Y
Lithobates catesbeianus	American bullfrog	N	131C	132T	202C	203R	220Y
Petromyzon marinus	Sea lamprey	N	143G	144S	215C	216P	233A

Single amino acid modifications selected to represent variations in other species at positions critical to enzyme catalytic function

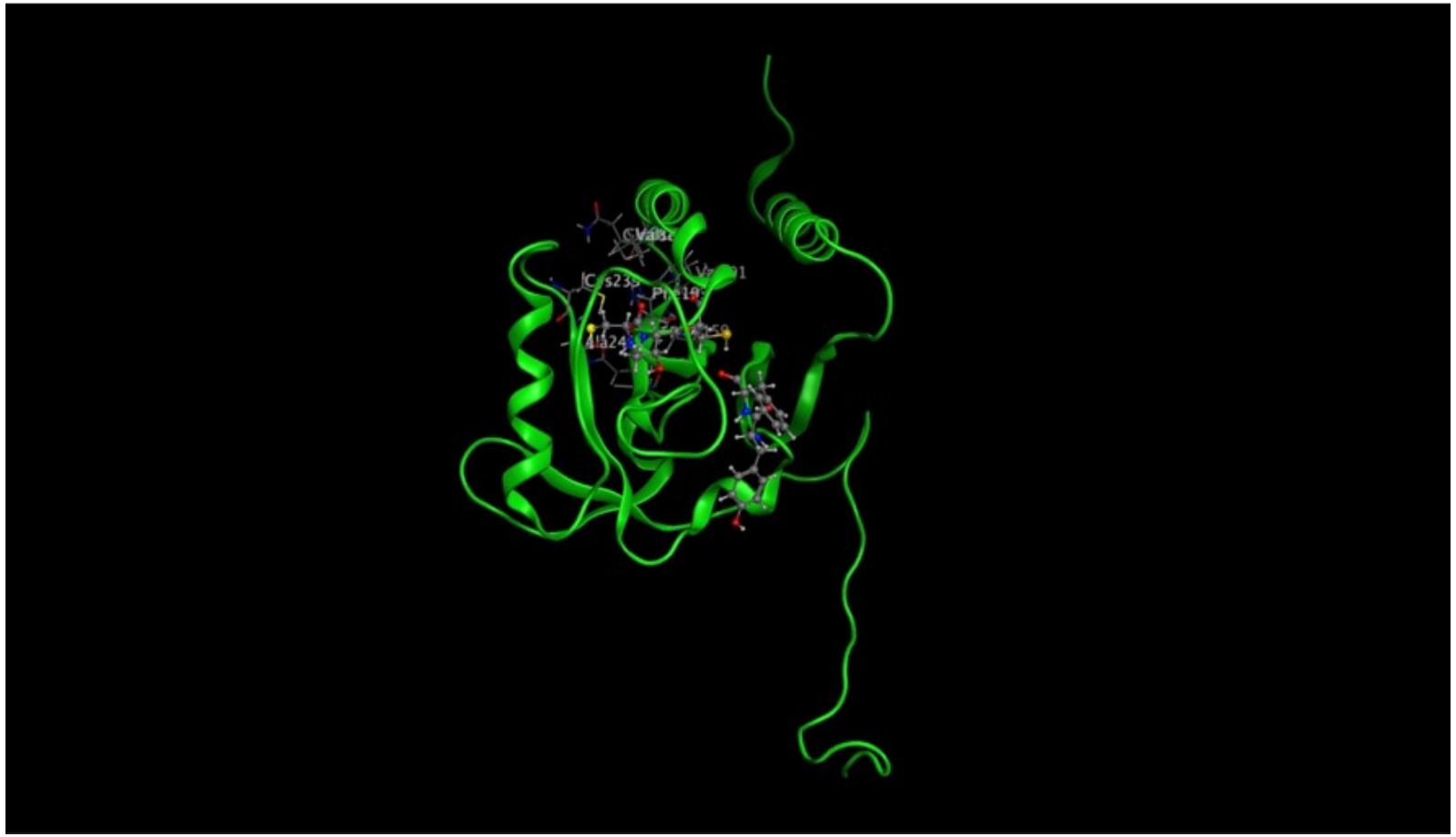


Molecular modeling

- Mouse dio3 crystal structure
  - Human homology model
- Catalytic (T4/T3 substrate binding) site
- Cofactor binding site
  - In vivo cofactor unknown (PRX?)
  - In vitro cofactor DTT (dithiothreitol)



Molecular Operating Environment (MOE) Chemical Computing Group



Video showing mutation locations

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Virtual docking affinity (S) scores through in-silico mutagenesis

Docking limitations:

- Solvent (water) often ignored by docking programs
- Lack of motion (ligand is flexible but protein is rigid)
- Interaction calculations are conducted with simple potential energy functions rather than more accurate quantum mechanics
- **Complexity of the type 3 deiodinase molecule!**

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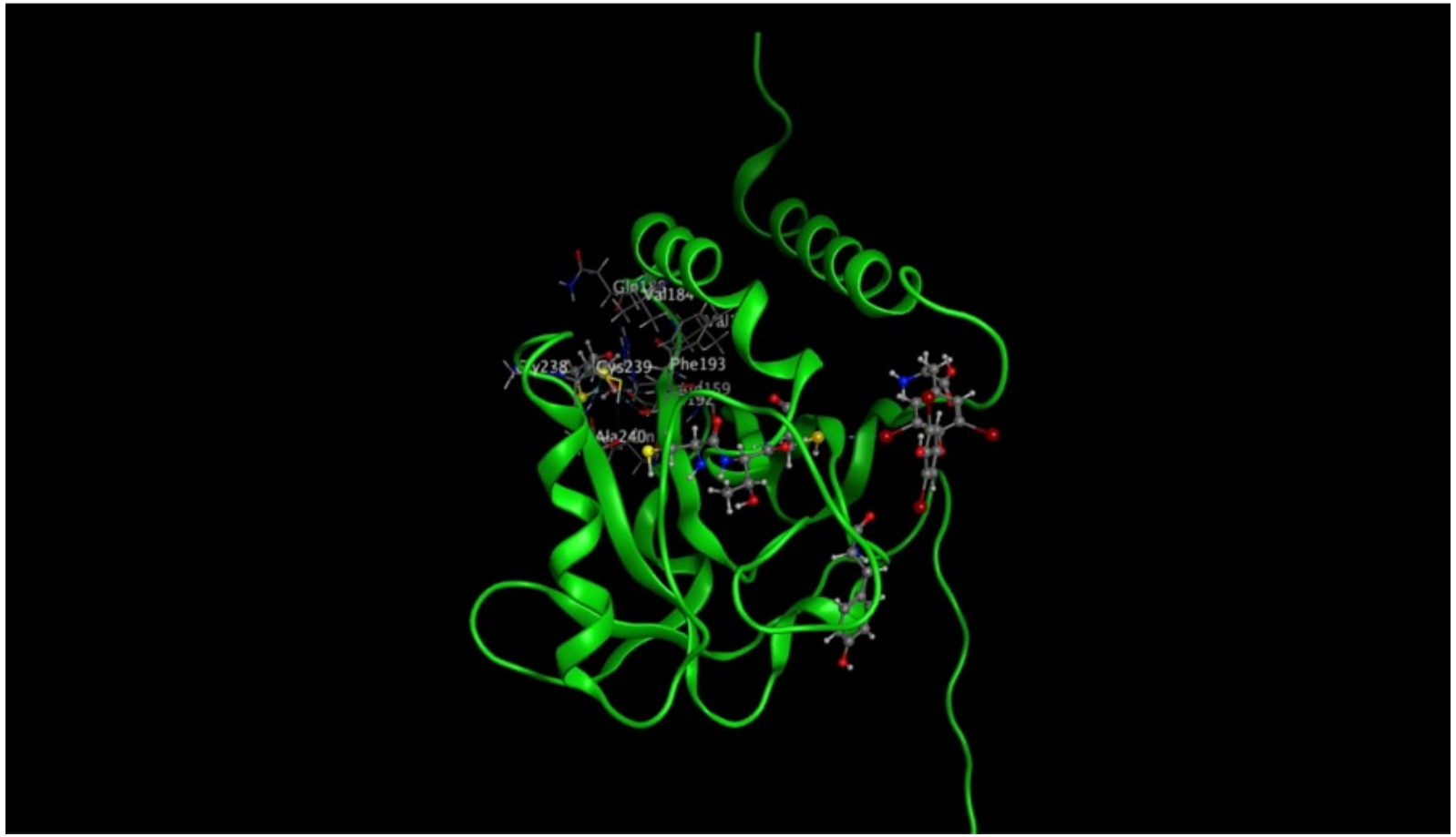
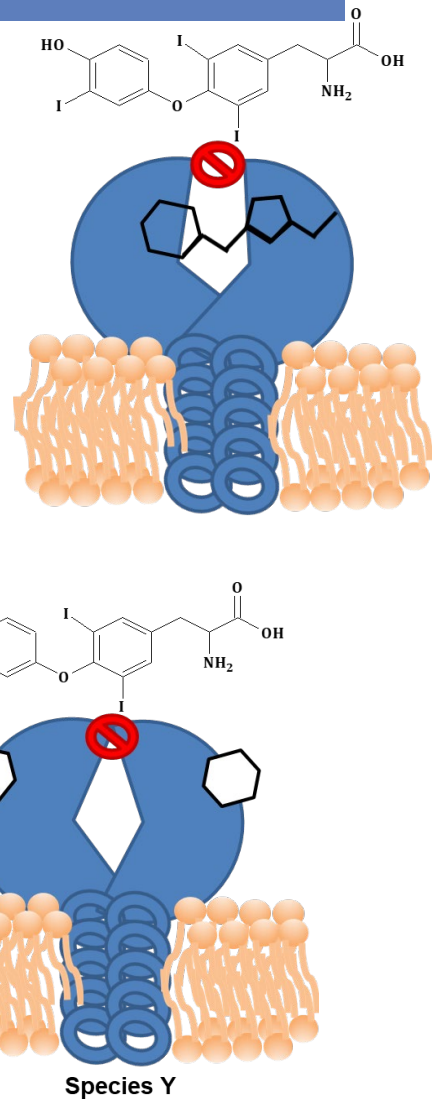
Molecular Operating Environment (MOE) Chemical Computing Group



Video showing kepone binding to cofactor location

Hypotheses for protein-ligand interactions

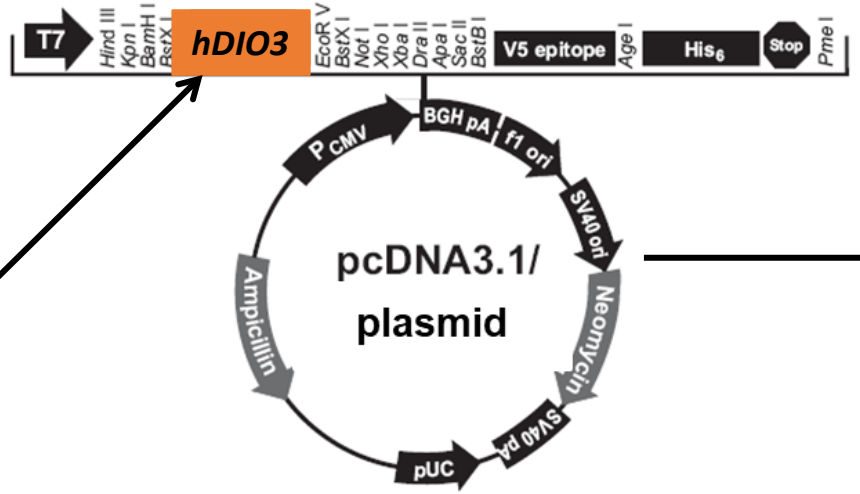
- Putative specific competitive inhibitors (Xanthohumol, Fiptonil)
  - Hypothesis: difference among catalytic site variants
    - C168G, T169S (catalysis) Y257A, Y257F (structure)
  - No difference among cofactor site variants
    - C239S, A240R (cofactor interaction and catalysis)
- Putative allosteric inhibitors (Kepone, NDGA)
  - Hypothesis: no difference among catalytic site variants
    - C168G, T169S (catalysis) Y257A, Y257F (structure)
  - Difference among cofactor site variants
    - C239S, A240R (cofactor interaction and catalysis)

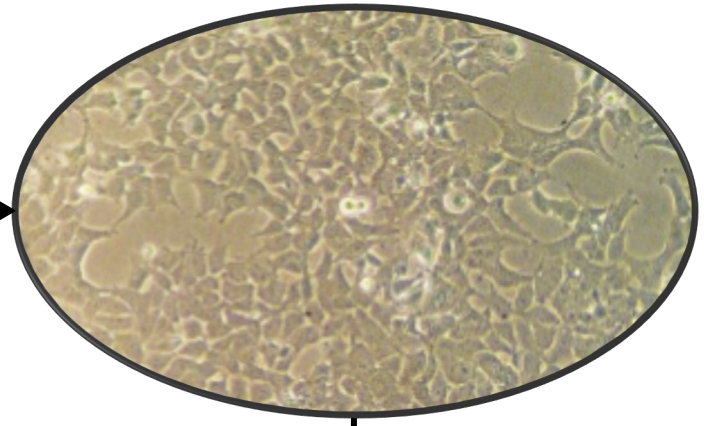


Protein expression & deiodinase in vitro screening assay methods

Transfect each SDM mutant plasmid construct into HEK293 cells.

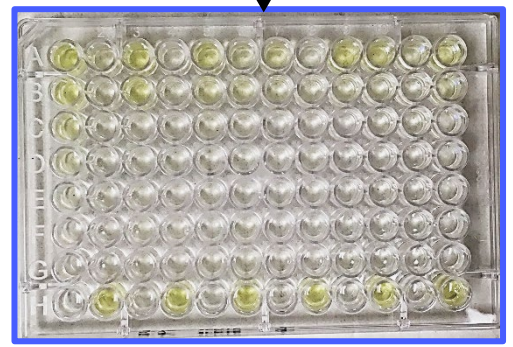
hDIO3 mutants:
C168G
T169S
C239S
A240R
Y257A
Y257F





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.



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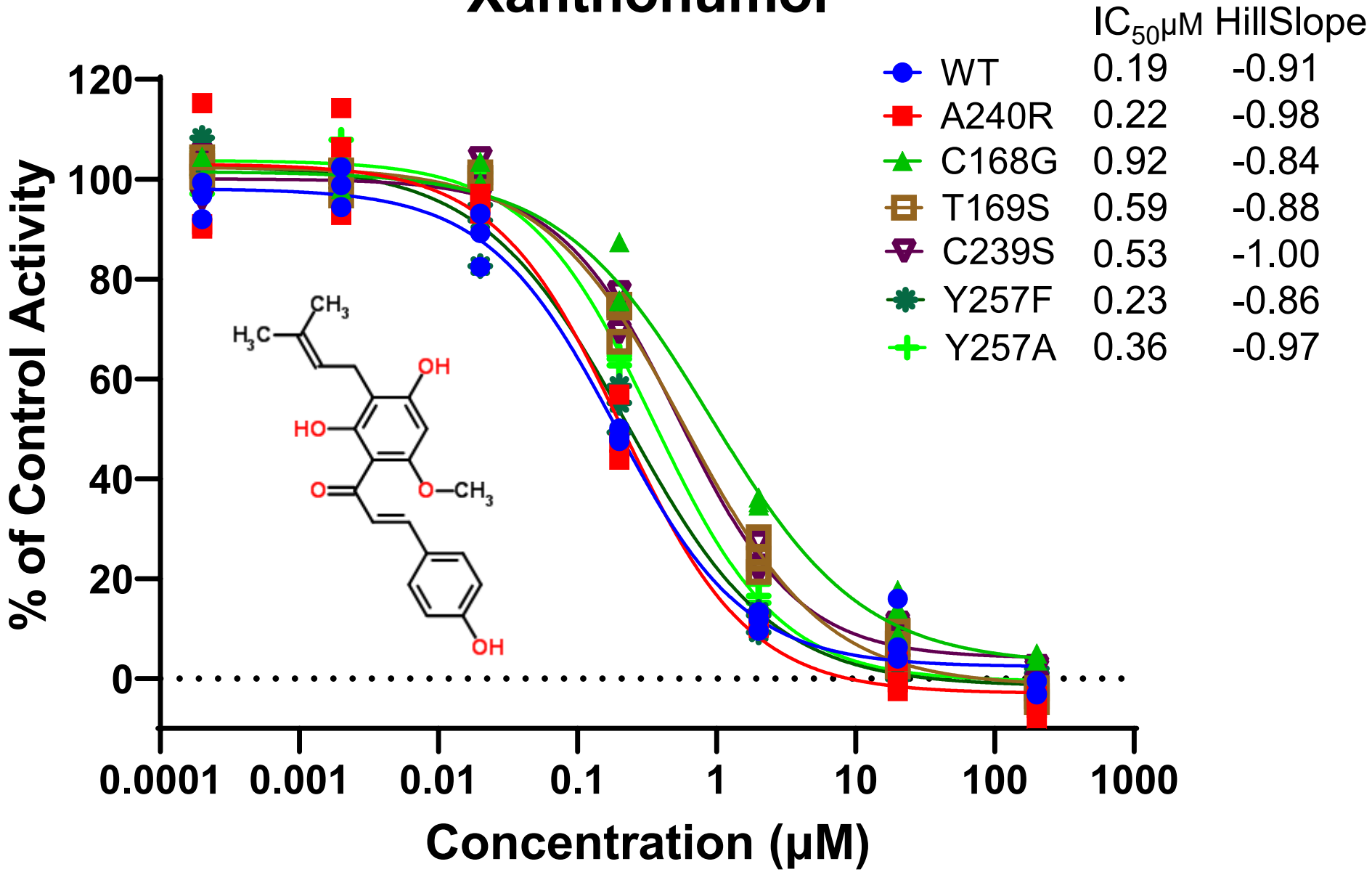
Xanthohumol



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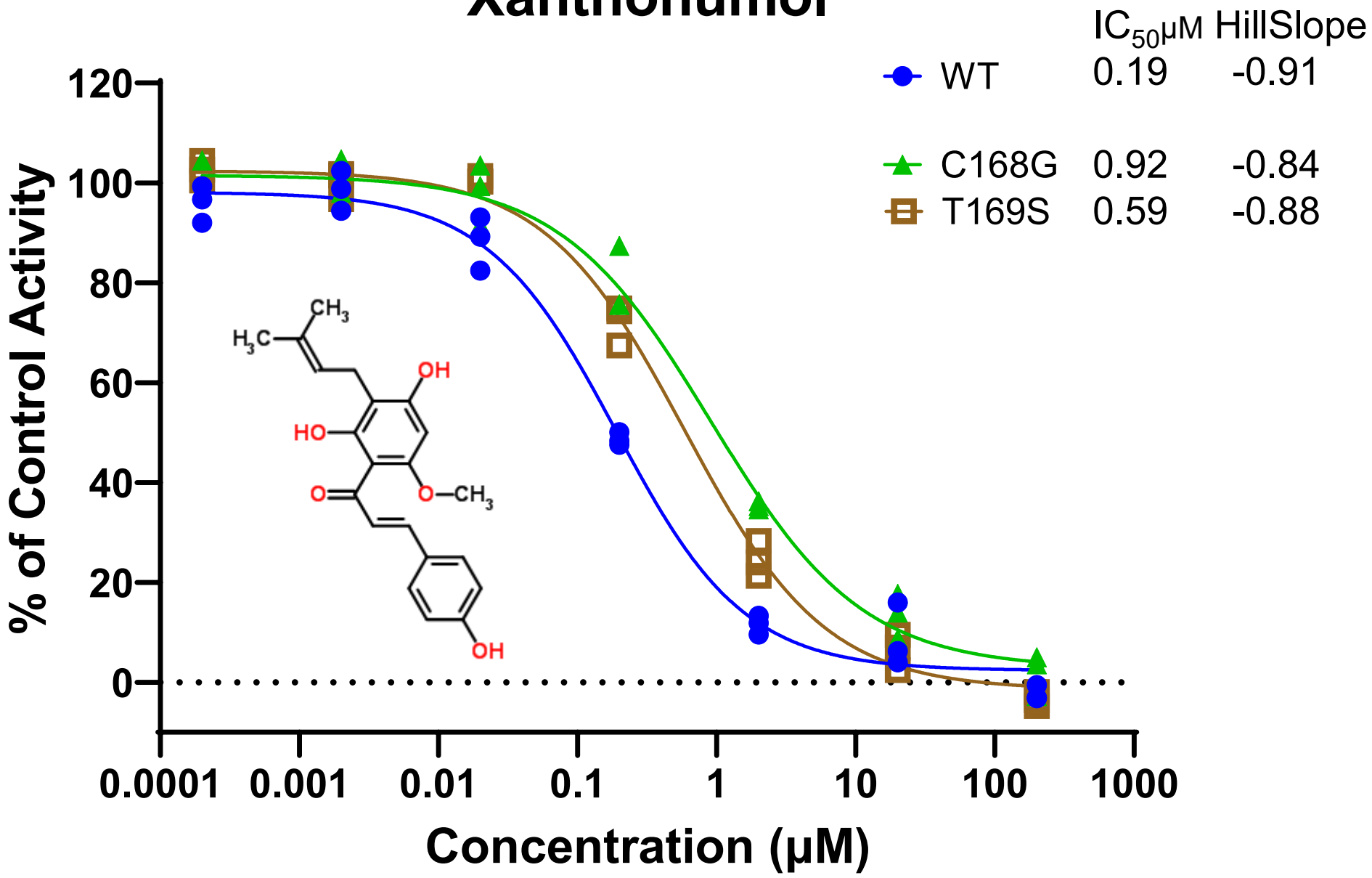
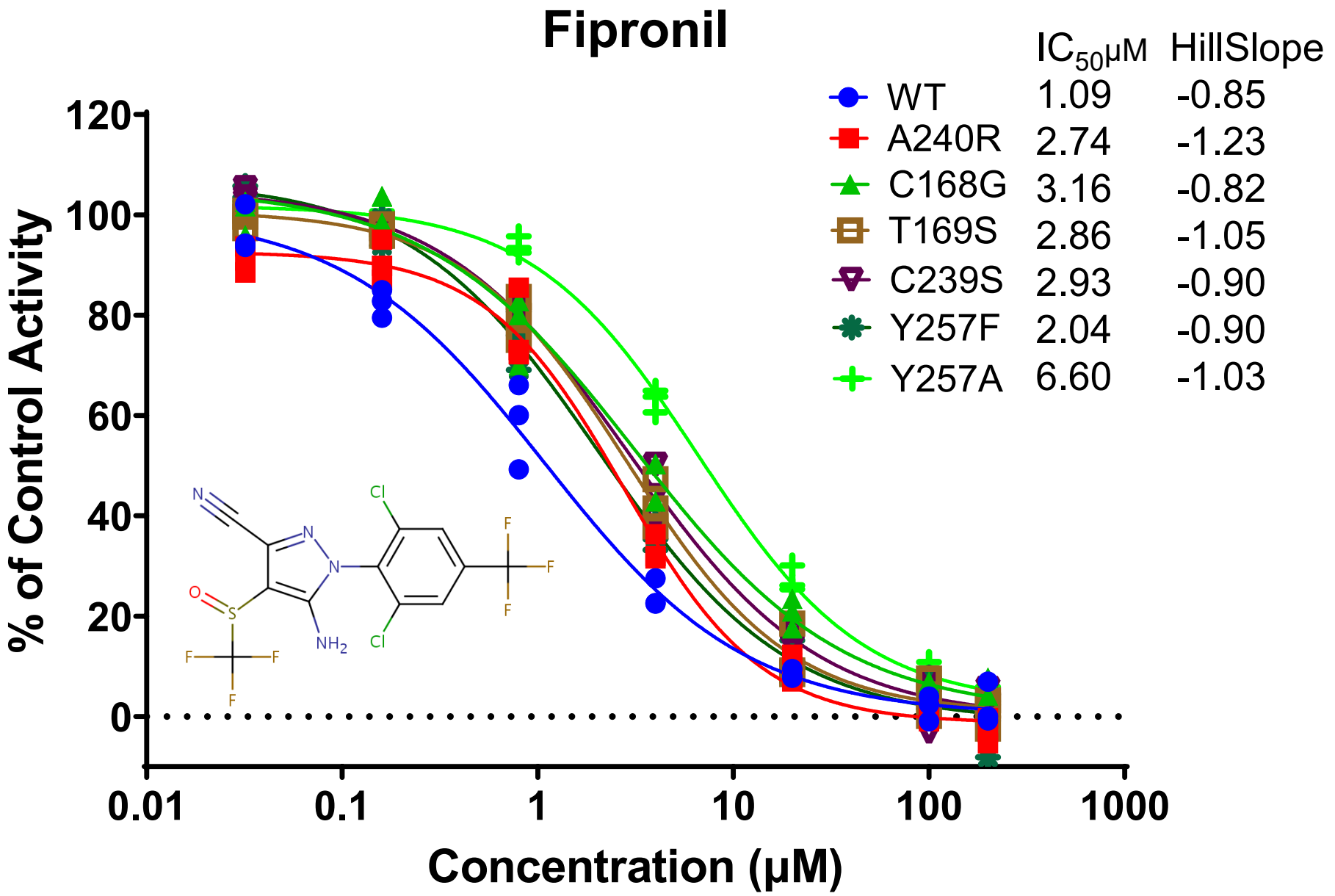
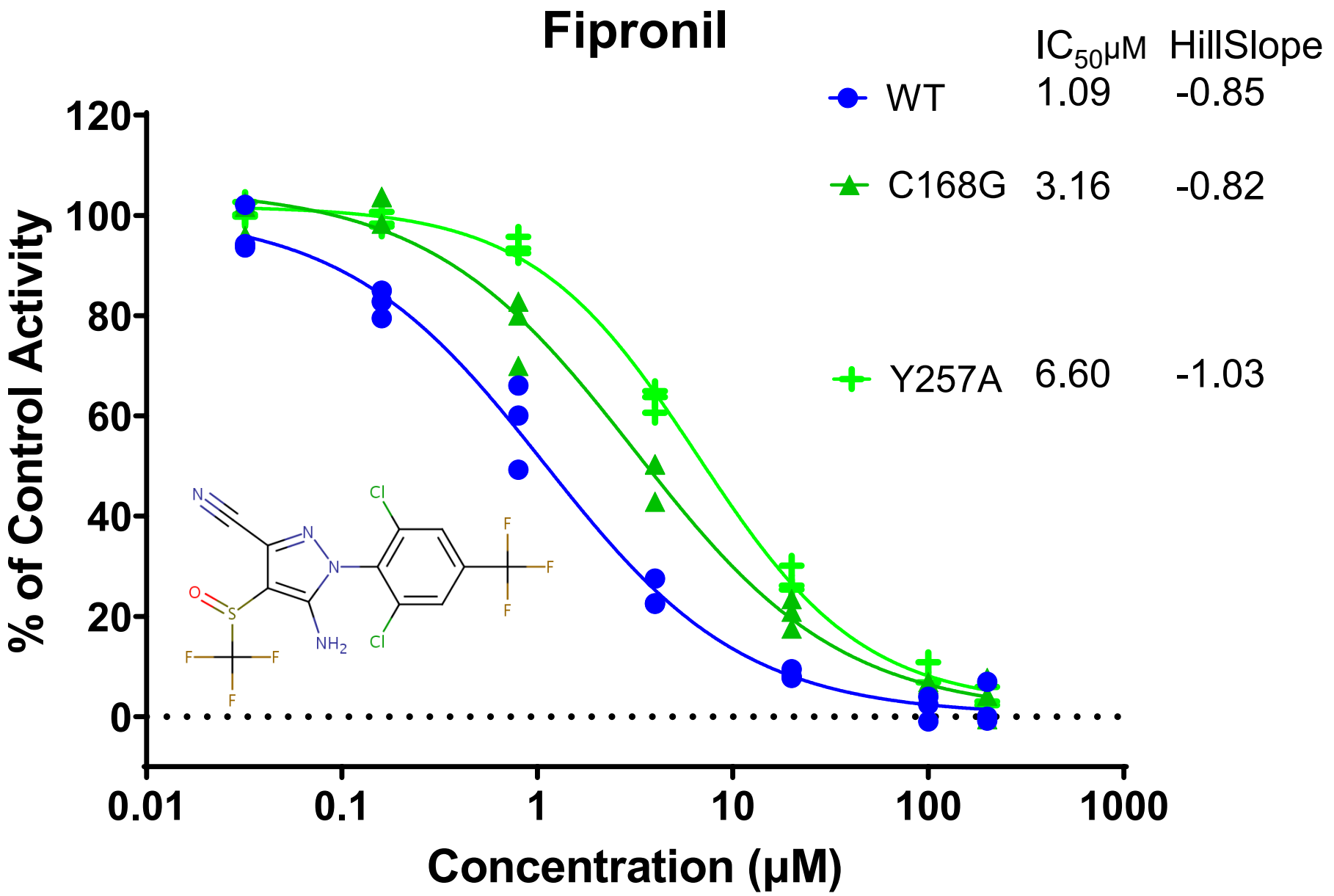
Xanthohumol




Fipronil
IC50μM HillSlope
WT 1.09 -0.85
A240R 2.74 -1.23
C168G 3.16 -0.82
T169S 2.86 -1.05
C239S 2.93 -0.90
Y257F 2.04 -0.90
Y257A 6.60 -1.03
% of Control Activity
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration (μM)
N
N
N
Cl
Cl
F
F
F
O
S
F
F
F
NH2


Fipronil
IC50μM HillSlope
WT 1.09 -0.85
C168G 3.16 -0.82
Y257A 6.60 -1.03
% of Control Activity
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration (μM)
N
N
N
Cl
Cl
F
F
F
O
S
F
F
F
NH2

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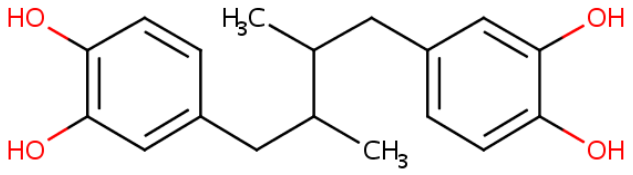
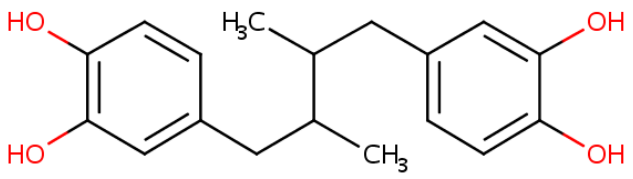
Nordihydroguaiaretic acid

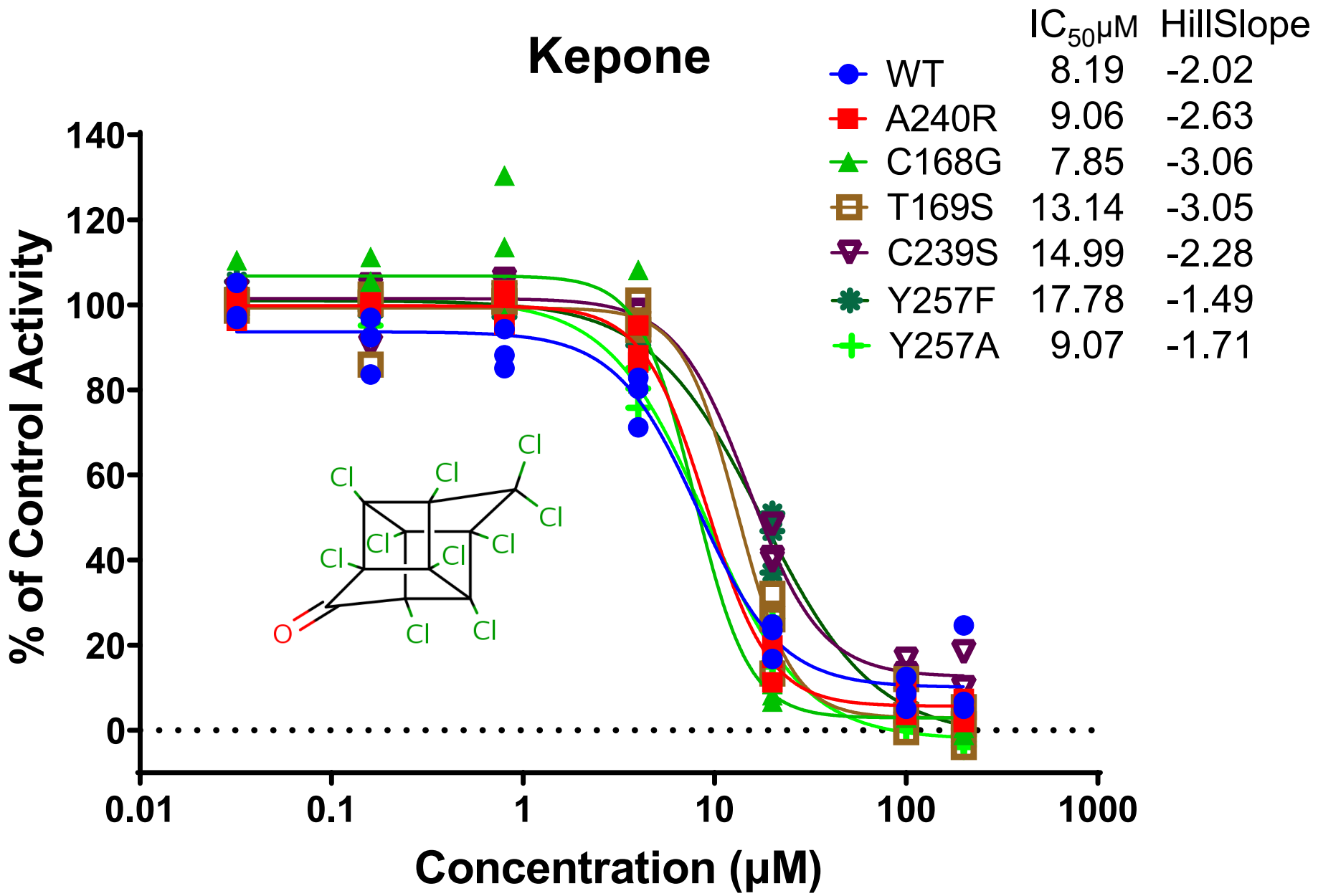


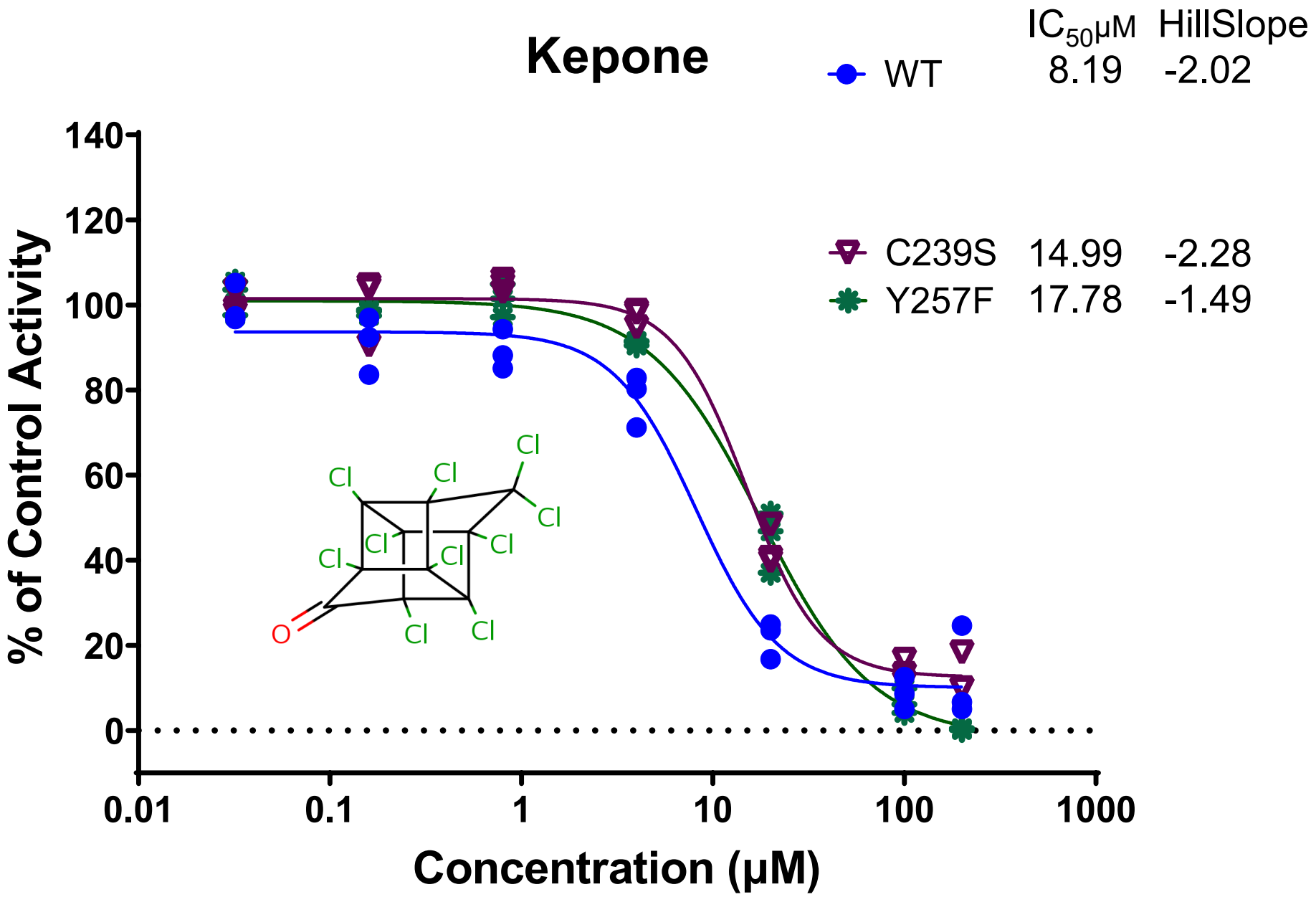
	IC_{50}μM	HillSlope
WT	0.27	-0.88
A240R	0.60	-0.92
C168G	1.38	-0.77
T169S	0.63	-1.12
C239S	1.07	-1.19
Y257F	0.45	-0.88
Y257A	0.18	-0.82

Nordihydroguaiaretic acid



	IC_{50}μM	HillSlope
WT	0.27	-0.88
C168G	1.38	-0.77
C239S	1.07	-1.19
Y257A	0.18	-0.82


Kepone
IC50μM HillSlope
WT 8.19 -2.02
A240R 9.06 -2.63
C168G 7.85 -3.06
T169S 13.14 -3.05
C239S 14.99 -2.28
Y257F 17.78 -1.49
Y257A 9.07 -1.71
% of Control Activity
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration (μM)
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
O


Kepone
IC50μM HillSlope
WT 8.19 -2.02
C239S 14.99 -2.28
Y257F 17.78 -1.49
% of Control Activity
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration (μM)
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
Cl
O

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Summary

- Small differences in IC_{50}s predicted by small differences in affinity scores
- A240R curves/IC_{50}s were similar to wildtype for all chemicals
- Putative specific competitive inhibitors (Xanthohumol, Fipronil)
  - Difference among catalytic site variants
    - C168G, T169S (catalysis), Y257F (structure)
- Putative allosteric inhibitors
  - NDGA: difference among catalytic and cofactor variants
    - C168G (catalysis), Y257A (Structure), & C239S (cofactor)
  - Kepone: difference for cofactor site and structure variants
    - C239S (cofactor interaction and catalysis) Y257F (structure)
- For theses chemicals: minor implications for species with these amino acid variations in the type 3 iodothyronine deiodinase enzyme

Acknowledgements

EPA/GLTED Bioinformatics team

Sara Vliet

Jenna Cavallin

Donovan Blatz

Carlie LaLone

EPA/GLTED Thyroid team

Philip DeGoey

Joe O'Flanagan

Mike Hornung

Sig Degitz

EPA, Durham, NC

Michael "Rocky" Goldsmith, computational chemistry (MOE)

This work was funded wholly by the U.S. Environmental Protection Agency.

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