The Many Cards Up 'Computational Sleeves': Reshaping In Silico Tools Used In Drug Discovery To Design Safer And Functional Chemicals

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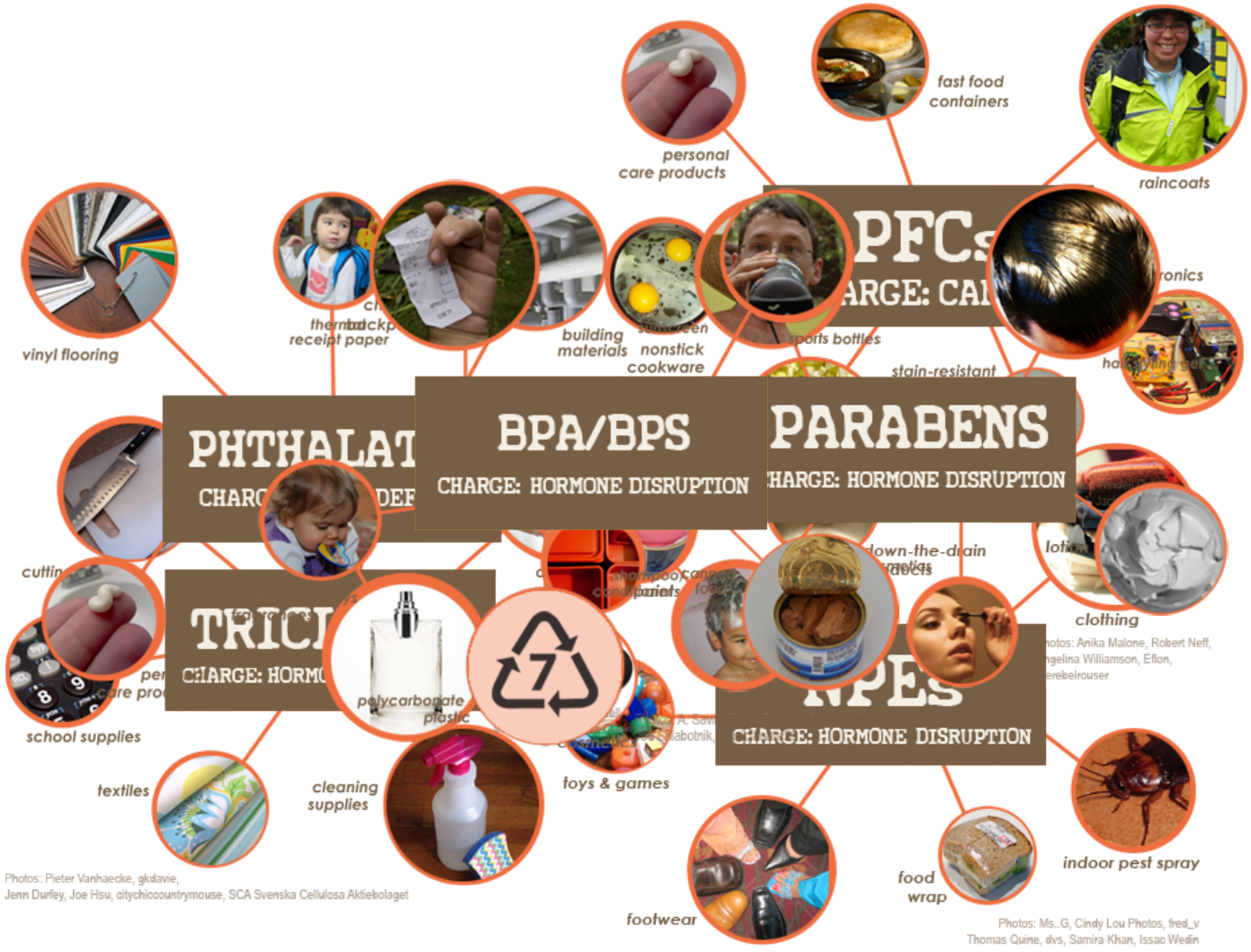
George Washington University


personal care products
fast food containers
raincoats
vinyl flooring
receipt paper
building materials
nonstick cookware
sports bottles
stain-resistant
PFCs
CHARGE: CA
PHTHALAT
CHARG
BPA/BPS
CHARGE: HORMONE DISRUPTION
PARABENS
CHARGE: HORMONE DISRUPTION
down-the-drain
clothing
TRICL
CHARGE: HORMO
polycarbonate plastic
school supplies
textiles
cleaning supplies
toys & games
NPES
CHARGE: HORMONE DISRUPTION
indoor pest spray
food wrap
footwear
Photos: Pieter Vanhaecke, gkdavie, Jenn Durfey, Joe Hsu, citychiccountrymouse, SCA Svenska Cellulosa Aktiebolaget
Photos: Anika Malone, Robert Neff, Angelina Williamson, Eflon, rebelrouser
Photos: Ms..G, Cindy Lou Photos, fresi_v Thomas Quine, dvs, Samira Khan, Issac Wedin

Alternative approach to alternatives assessment

Reactive Approach

- identify hazardous chemicals from those already in existence
- evaluate chemical alternatives
- hazard assessment

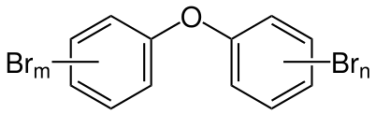
Proactive Approach

- Design a new chemical that has a superior safety profile to chemicals in the market
- Redesign an existing chemical to minimize biological activity
- **Requires that chemists consider biological activity alongside function at the design stage**

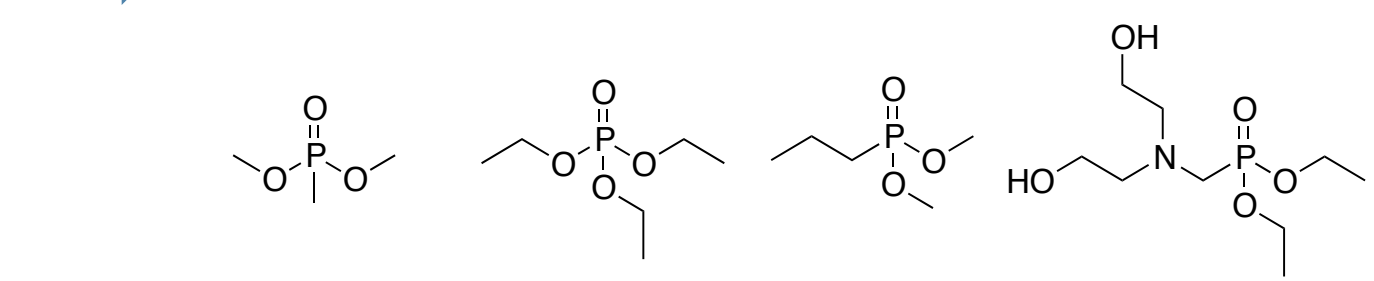
"Chemical products should be designed to affect their desired function while minimizing their toxicity" (4th principle of Green Chemistry)

Anastas, P. and Warner, W. *Green Chemistry: Theory and Practice,* Oxford University Press: New York, 1998.

Case study: Organophosphorus flame retardants



Polybrominated diphenyl ethers

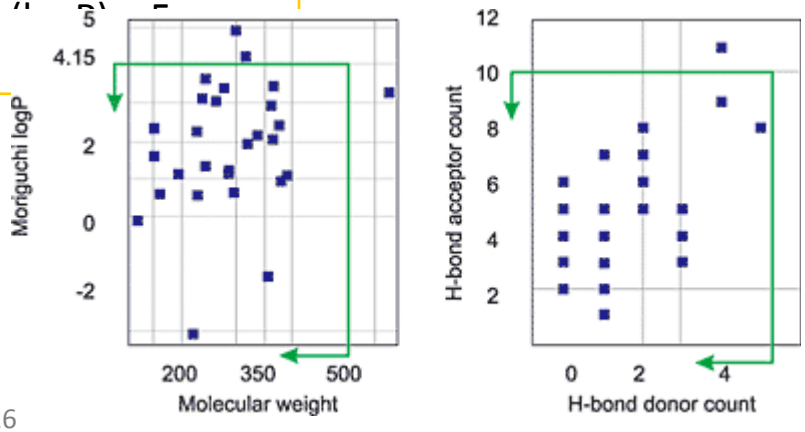


Lipinski's rule of five for drug likeness

Lipinski, 1997
~90% of drugs on the market have the following properties in common:

Lipinski's Rule of Five

1. Not more than 5 hydrogen bond donors
2. Not more than 10 hydrogen bond acceptors
3. Molecular weight under 500 D
4. Octanol-water coefficient



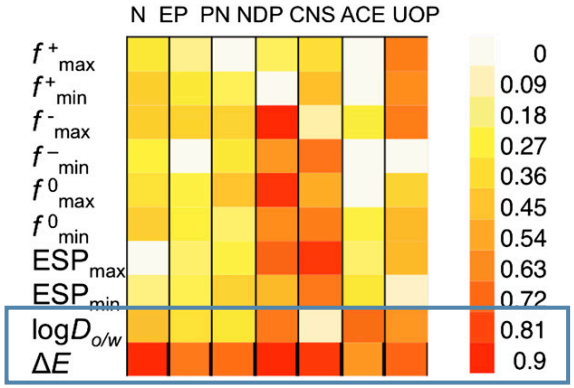
Lipinski, C. et al, *Adv. Drug Del. Rev.* 2001, 46: 3–26

Rule-based safer chemical design for acute and chronic aquatic toxicity of commercial chemicals

- Based on 555 compounds tested in Fathead minnow (US EPA, validated and diverse dataset)
- Data group by category of concern:

Category	Level of concern	Lower limit (LC_{50}, mmol/L)	Upper limit (LC_{50}, mmol/L)	Number of compounds
1	High	0	0.0067	81
2	Moderate	0.0067	1.49	305
3	Low	1.49	3.32	80
4	None	3.32	—	89
			Total	555

- Descriptor focus on **bioavailability** (partition/ distribution coefficients) and **reactivity** (FMO Theory):



N = narcosis;
EP = electrophiles;
PN = polar narcosis;
NDP = neurodepressant;
CNS = central nervous system seizure or stimulant;
ACE = acetylcholinesterase inhibitors;
UOP, uncoupler of oxidative phosphorylation.

Fukui indices:

$$f_A^+ = p_A(N+1) - p_A(N)$$

$$f_A^- = p_A(N) - p_A(N-1)$$

$$f_A^0 = \frac{1}{2}\left[\left(p_A(N+1) - p_A(N-1)\right)\right]$$

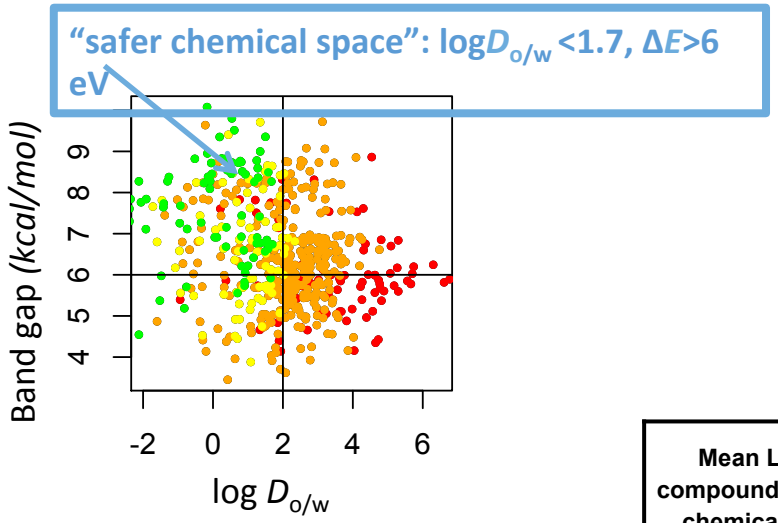
mPW1PW91/MIDIX+

Voutchkova, A.; Kostal, J.; Steinfeld, J.; Emerson, J. W.; Brooks, B. W.; Anastas, P.; Zimmerman, J. B. Towards rational molecular design: derivation of property guidelines for reduced acute aquatic toxicity. *Green Chem.* 2011, 13, 2373-2379.

Kostal, J.; Voutchkova, A.; Zimmerman, J. B.; Anastas, P. Identifying and Designing Chemicals with Minimal Acute Aquatic Toxicity. *Proc. Natl. Acad. Sci. USA* Early edition 2015, *112*, 6289-6294

Rule-based safer chemical design for low ecotoxicity

- Chemicals in "safer space" **10x more likely to have low or no toxicity** to aq species than chemicals 'outside'

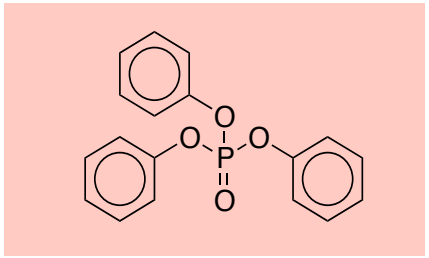


	Acute Aquatic Toxicity Concern Category	High	Moderate	Low	None	Mean LC_{50} of compounds in safer chemical space (mg/L)	(mmol/L)
Property-based filter	none	15%	55%	15%	15%	999	0.155
	log$D_{o/w}$<1.7	12 %	27%	80%	100%	2247	1.29
	log$D_{o/w}$<1.7; ΔE>6 eV	7 %	15 %	48%	89%	3006	2.71
	log$D_{o/w}$<1.7; ΔE>6 eV; V < 620 Å^3	1 %	11 %	45 %	88 %	3405	3.65

Kostal, J.; Voutchkova, A.; Zimmerman, J. B.; Anastas, P. Identifying and Designing Chemicals with Minimal Acute Aquatic Toxicity. *Proc. Natl. Acad. Sci. USA* 2015, *112*, 6289-6294

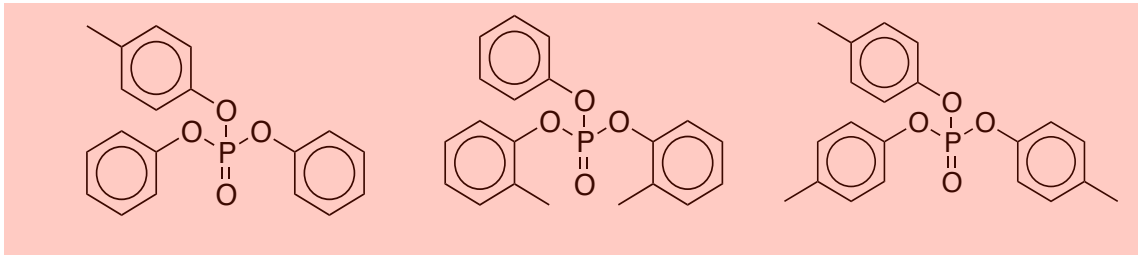
Application to OP flame retardants

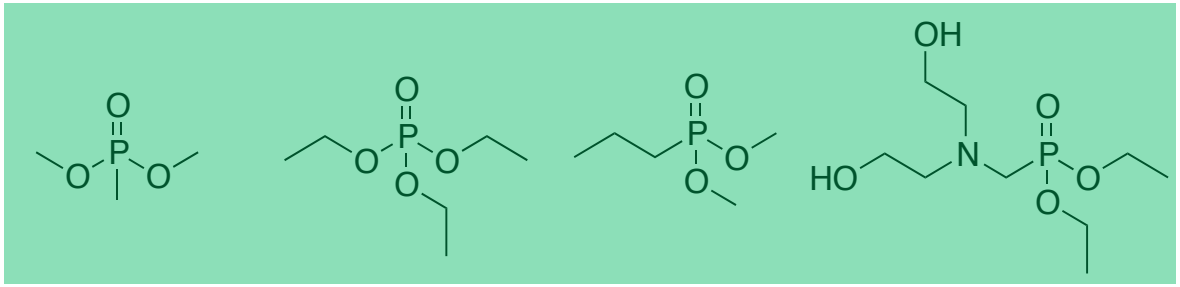
- Only aliphatic OP compounds were identified as safe to aquatic species



Triphenyl phosphate

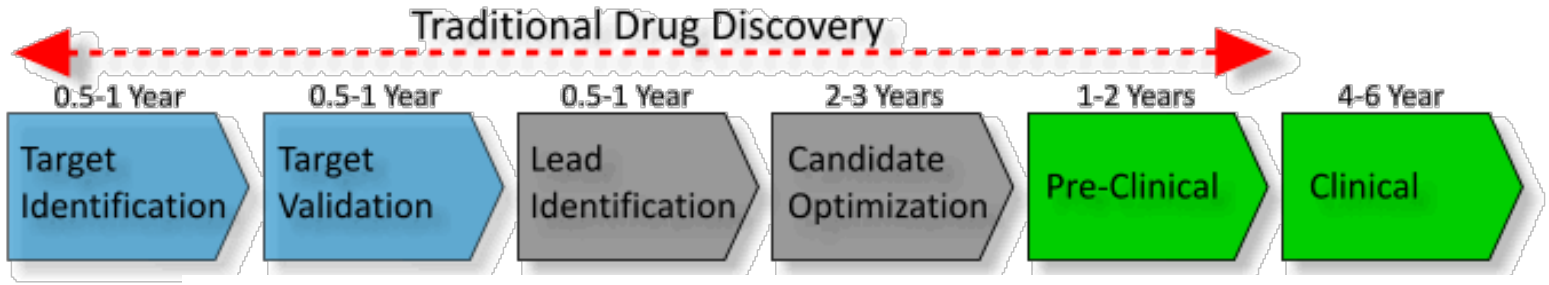
LC_{50}: 0.25 (High tox)

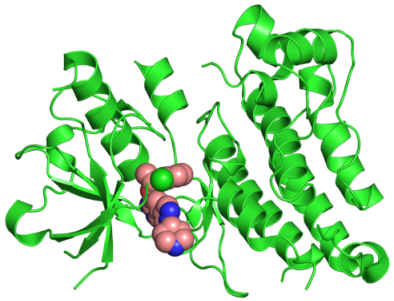




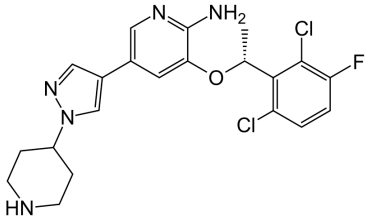
LC_{50}: 350 (low/no tox)

Target-specific design approaches





Target: Anaplastic Lymphoma Kinase

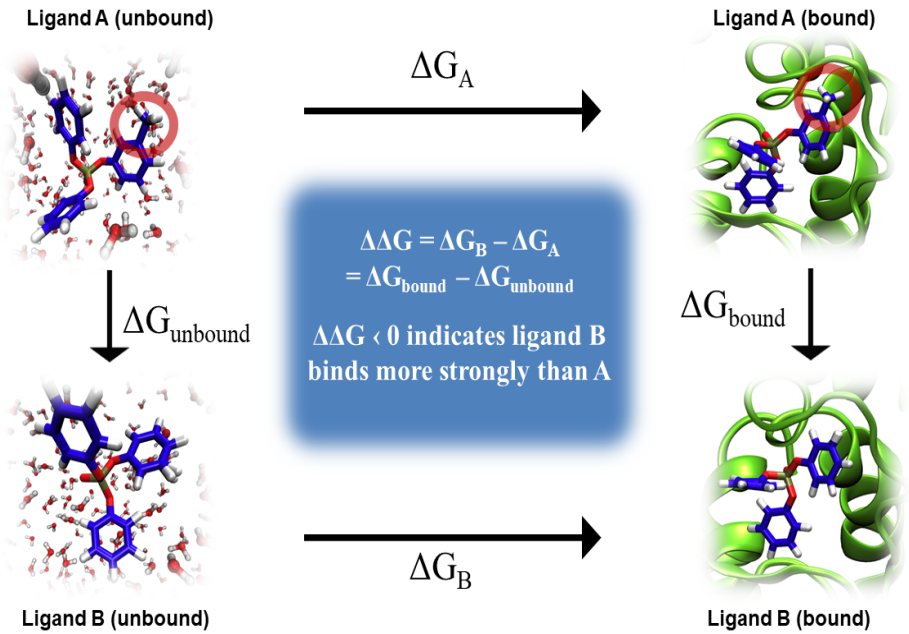


Optimized Lead

Marketed Drug

Statistical Free Energy Perturbation (FEP) calculations in safer chemical design

$$\Delta G(A \rightarrow B) = G_B - G_A = -k_B T \ln \left\langle \exp\left(-\frac{E_B - E_A}{k_B T} \right) \right\rangle_A$$

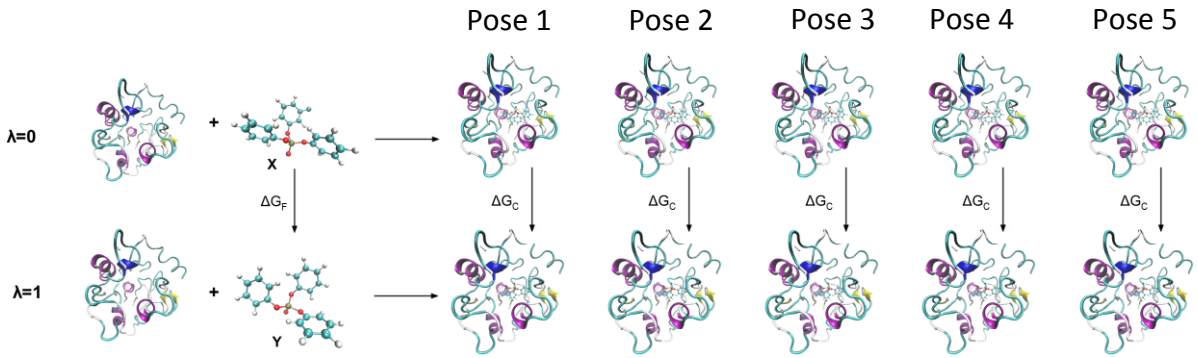


Kostal, J. *Computational Chemistry in Predictive Toxicology: status quo et quo vadis?* Advances in Molecular Toxicology 10,

Transforming MC/FEP to toxicant redesign

- **Challenges: multiple targets and difficult to prove a negative**

  → solution: larger computational resources and
  "Multiple-pose 2D Free Energy Perturbation calculations"

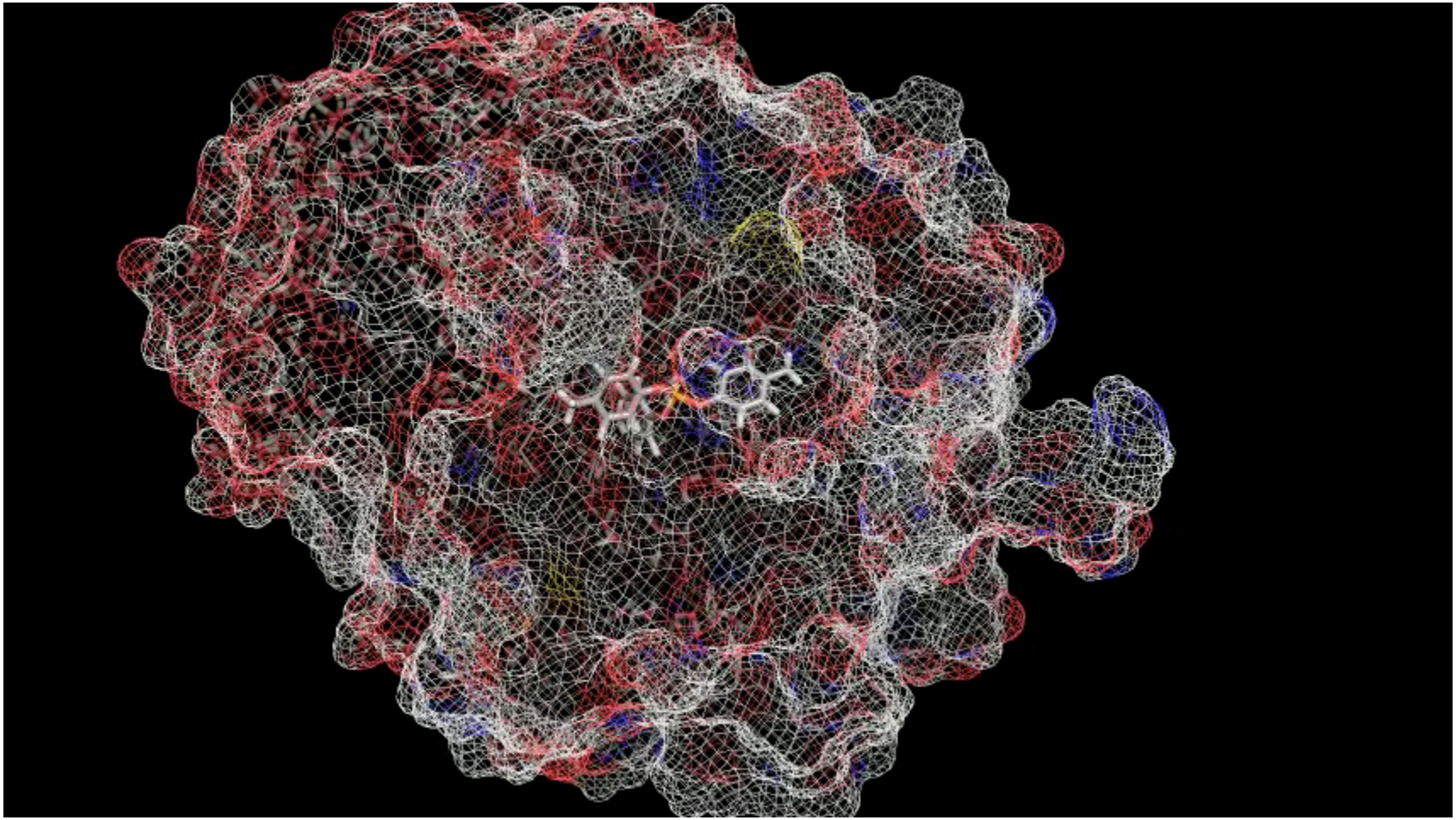


$$\Delta\Delta G_{\mathrm{cor}} = -kT \ln\left(\frac{\exp\left(-\beta\Delta\Delta G_{\mathrm{pose1}}\right) + \exp\left(-\beta\Delta\Delta G_{\mathrm{pose2}}\right)}{2}\right)$$

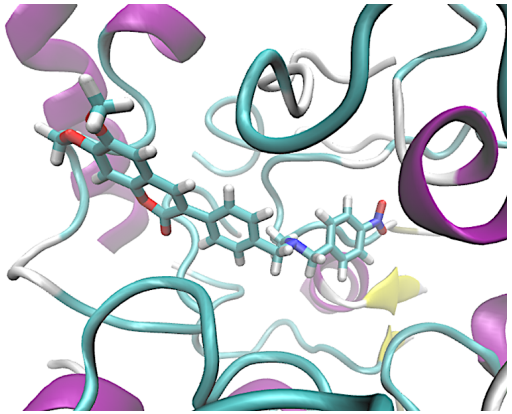
Select the **most unfavorable substitution**, considering the most favorable of equivalent analogs (in terms of FEP direction or ring position due to rotation)

Average energetically-equivalent poses that are too different to be sampled:

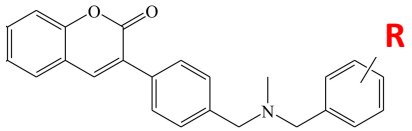
Kostal, J. *Computational Chemistry in Predictive Toxicology: status quo et quo vadis?* Advances in Molecular Toxicology 10,



Method validation: AChE

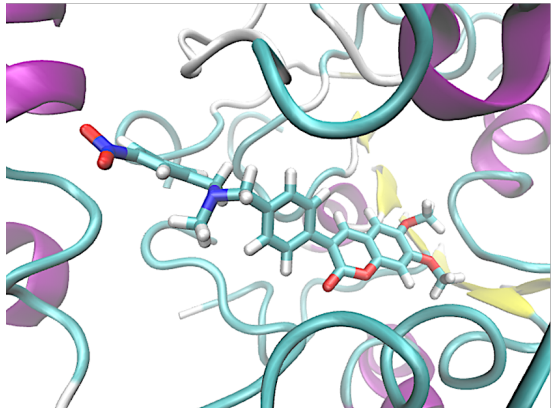


Perturbing position of substituents on inhibitor scaffold:

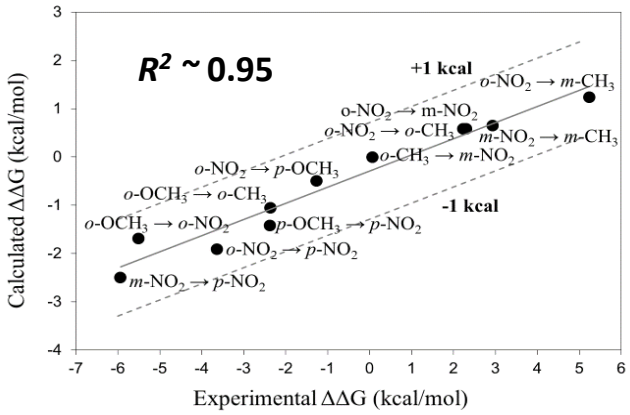


$\Delta\Delta G_{X\rightarrow Y} = RT\ln(IC_{50}{}^{X}/IC_{50}{}^{Y})$

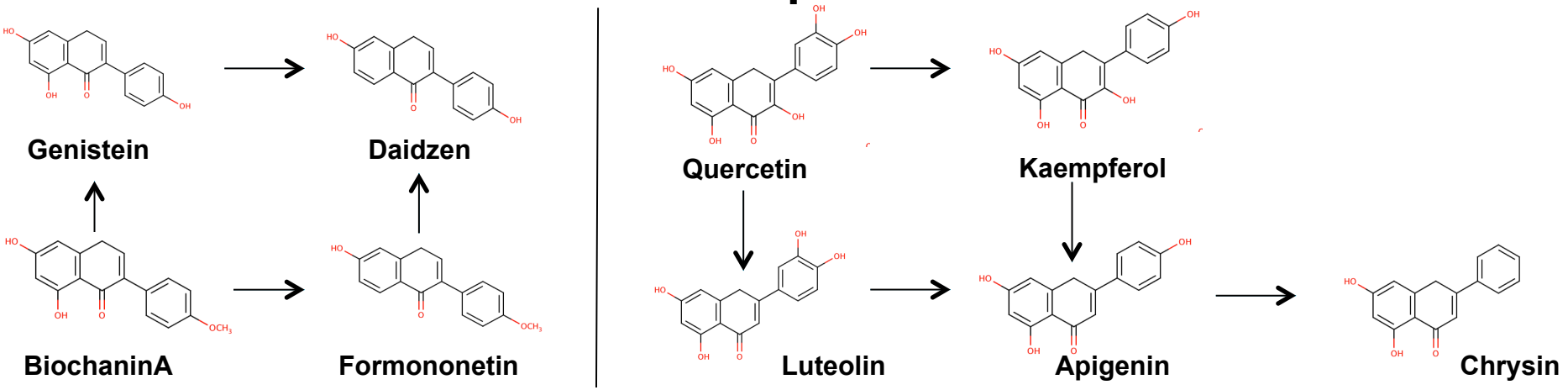
R = NO_2, CH_3,OCH_3

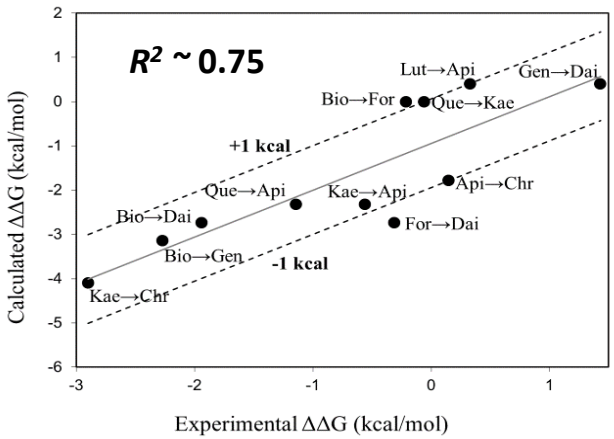


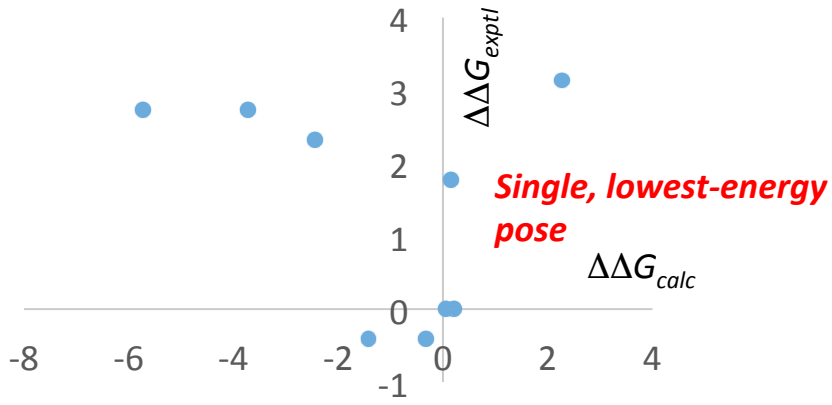
The accuracy in determining relative binding affinity using our MC/ FEP protocol with OPLS-AA/CM1A/TIP4P force field (R^2 > 0.90) greatly exceeds that of docking software force fields (R^2 ~ 0.1).



Method validation: ERβ

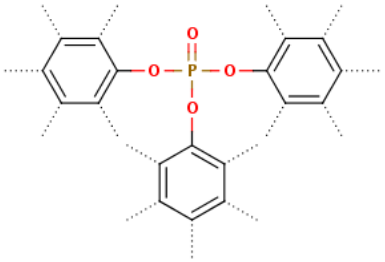


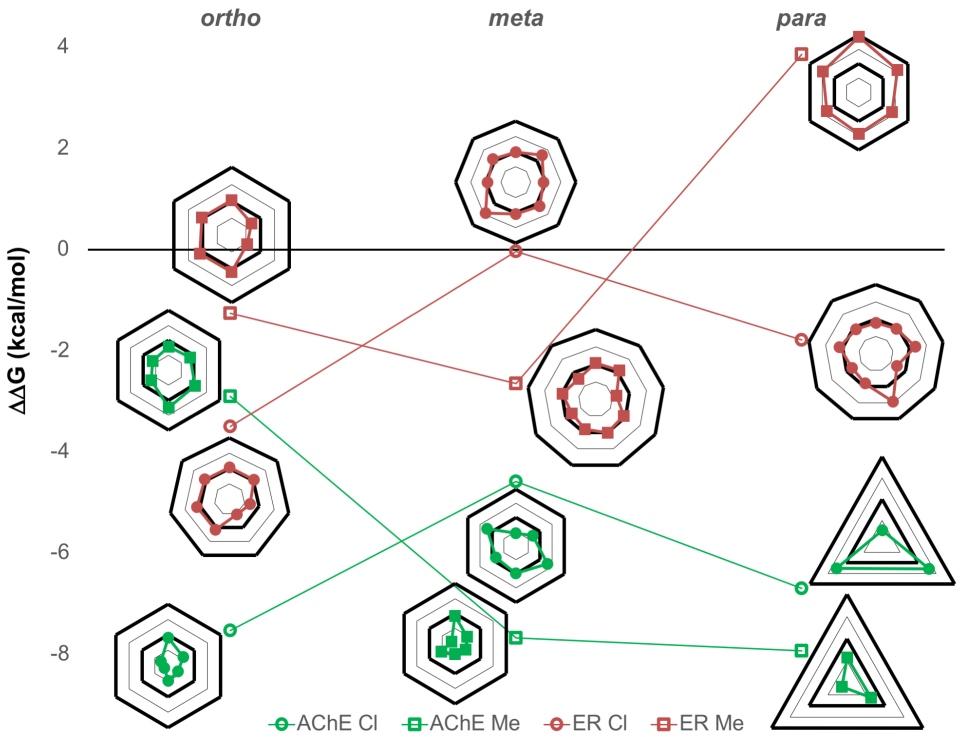




Redesigning TPP

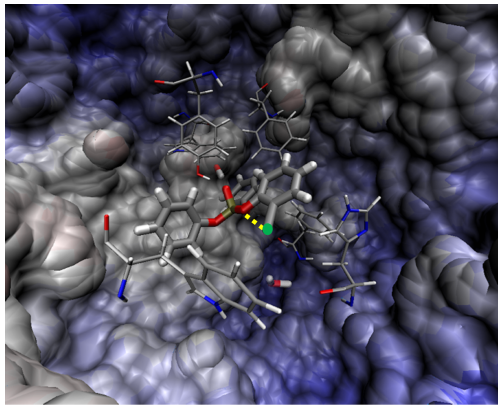
- CH_3 and X scans (to gauge desirable electronic nature of ring substitutions)

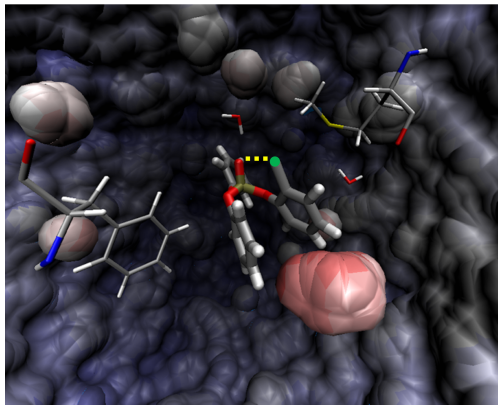




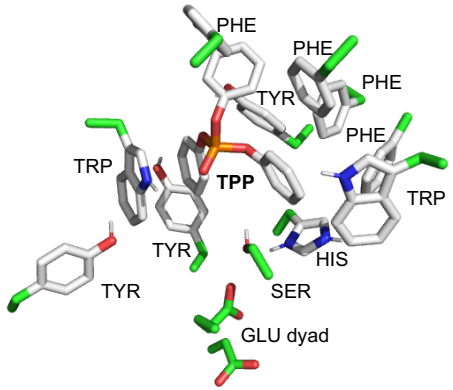
Halogen- and methyl-substituted TPP analogs

***ortho*-chloro-TPP bound to AChE**

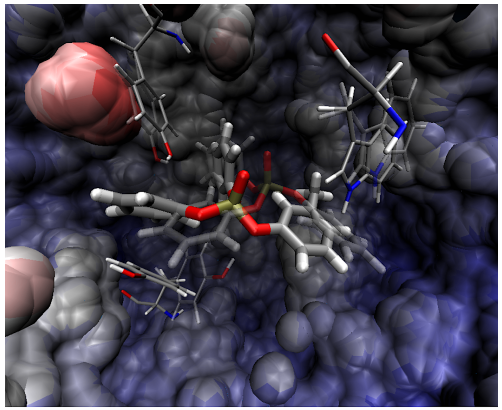


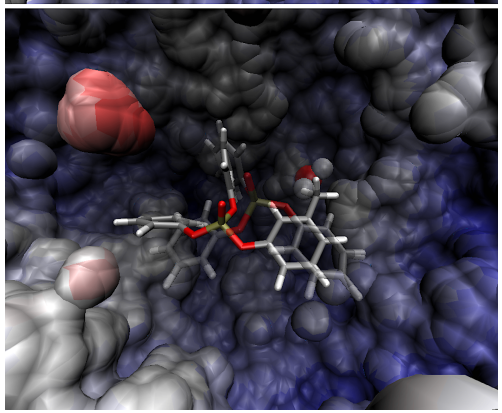


***ortho*-chloro-TPP bound to ER**



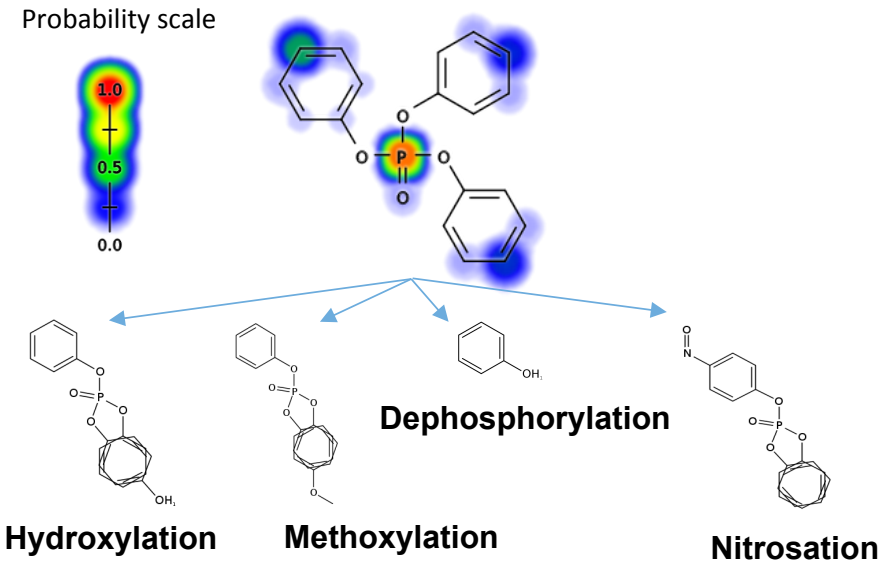
***meta*-methyl-TPP bound to AChE**



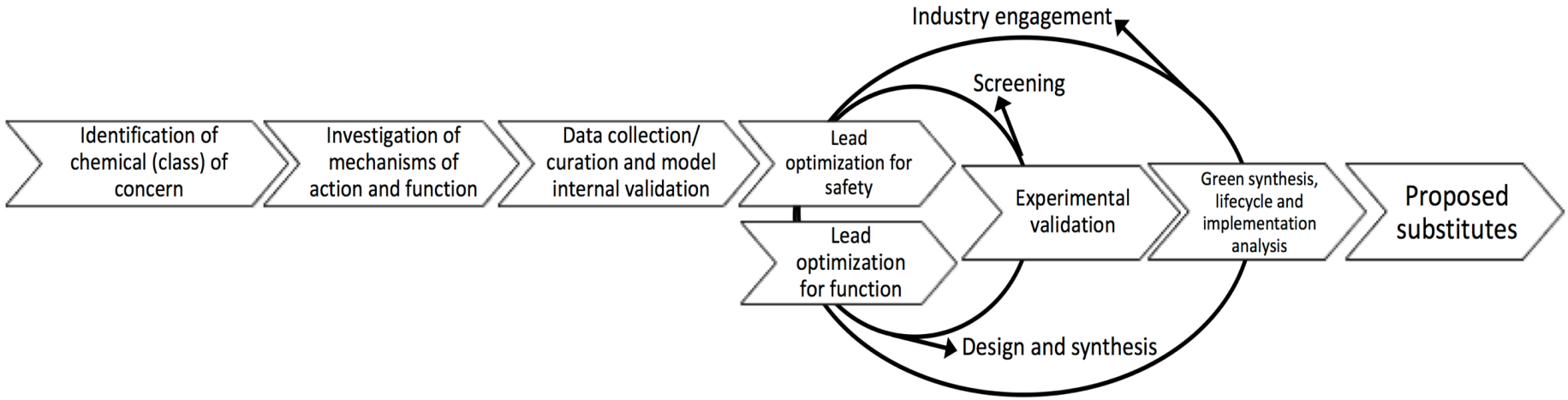


Tradeoffs

- Metabolism (bioaccumulation/persistence vs. degradation to a more potent analog)
- Function: halogenations enhances flame retardant function (gas-phase mechanism), methylation does not affect it – we can predict this computationally via calculations of oxidative and thermal stability
- Other targets, other pathways…



Incorporating *in silico* safer chemical design into development of safer alternatives



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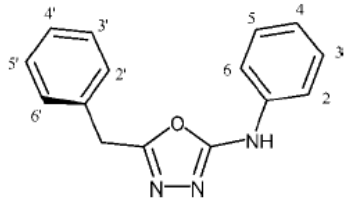
- Preston Griffin (GS)
- Matthew Winfough (GS)
- Robin Kleinberg (US)
- Selene Ramer (US)
- Sam Vaccaro (US)

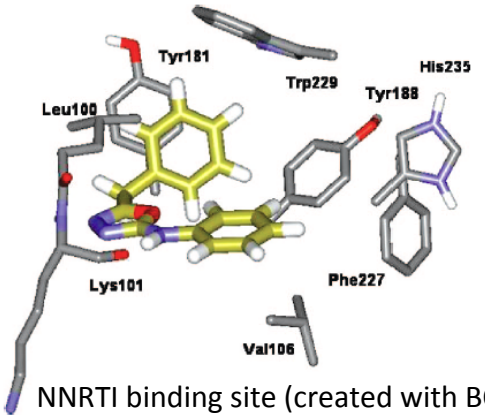
Support:

Collaborators:

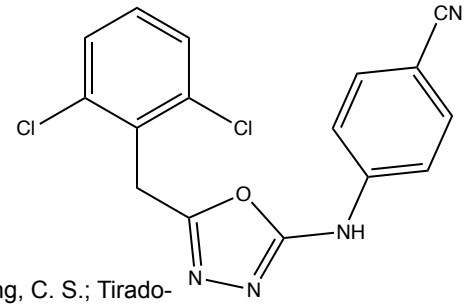
Example: Optimization of Azoles as Anti-Human Immunodeficiency Virus Agents



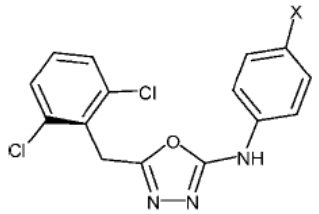
Cl → H	ΔG_{bound} (kcal/mol)	ΔG_{un} (kcal/mol)	$\Delta\Delta G_b{}^a$ (kcal/mol)	σ^b
C2	2.71	0.51	2.21	0.10
C3	1.33	−2.00	3.33	0.11
C4	6.62	2.42	4.20	0.09
C5	−0.54	−1.71	1.17	0.10
C6	−6.86	−4.26	−2.60	0.12
C2′	4.86	1.43	3.43	0.08
C3′	−4.01	0.69	−4.70	0.11
C4′	1.01	1.81	−0.80	0.09
C5′	1.71	0.80	0.91	0.08
C6′	4.89	1.12	3.77	0.08



NNRTI binding site (created with BOMB and optimized with OPLS-AA force field)



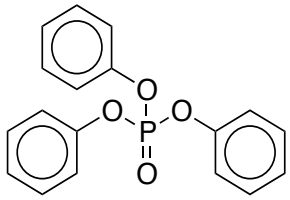
22 nM

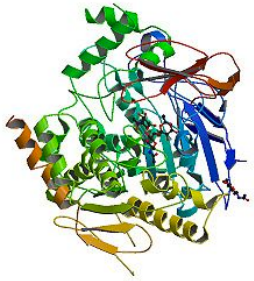


X →	Y	ΔG_{bound} (kcal/mol)	ΔG_{un} (kcal/mol)	$\Delta\Delta G_b{}^a$ (kcal/mol)	σ^b
CN	Cl	1.53	0.34	1.19	0.13
Cl	F	1.22	−0.54	1.75	0.05
F	H	5.56	3.27	2.29	0.05
Cl	H	6.99	2.23	4.76	0.06
CH_3	H	5.49	3.88	1.61	0.12
CH_3CH_2	CH_3	−3.27	−1.93	−1.34	0.13
CH_3CH_2	OCH_3	−6.46	−4.91	−1.55	0.16
CF_3	CH_3	−10.01	−10.63	0.62	0.08
CH_3OCH_2	CH_3CH_2	0.79	−0.63	1.41	0.17

Zeevaart, J. G.; Wang, L.; Thakur, V. V.; Leung, C. S.; Tirado-Rives, J.; Bailey, C. M.; Domaoal, R. A.; Anderson, K. S.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2008**, *130*, 9492-9499.

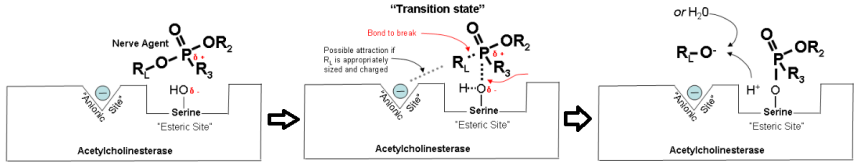
Transforming MC/FEP to toxicant redesign

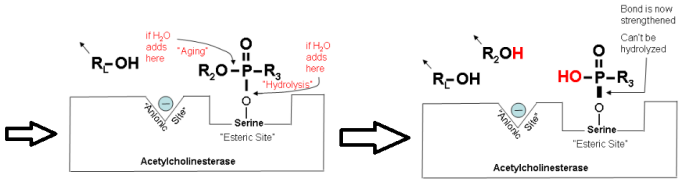


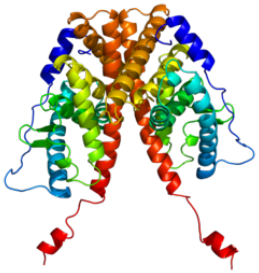


Acetylcholinesterase → Neurotoxicant

Electrostatic binding and/or covalent (phosphorylation of SER)







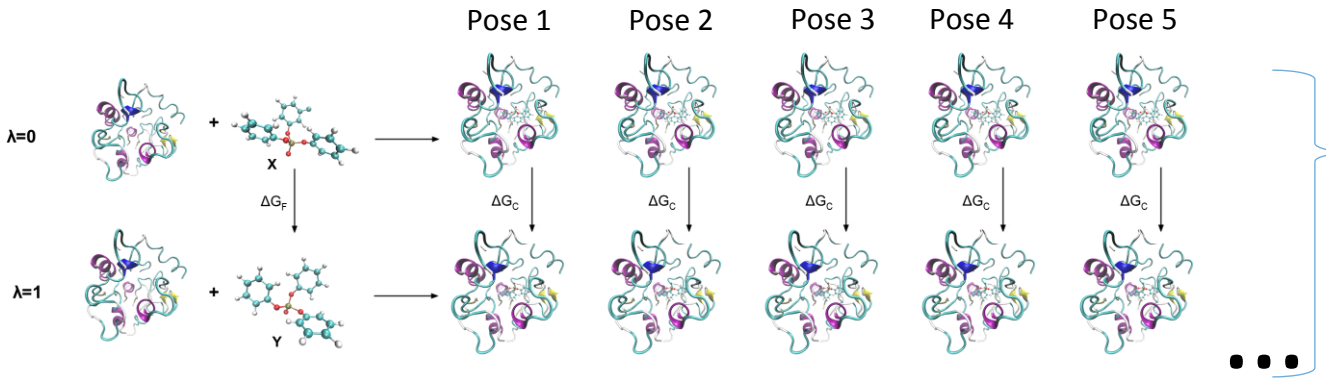
Estrogen receptor α → Endocrine disruptor

Electrostatic binding

Transforming MC/FEP to toxicant redesign

- **Challenges: multiple targets and difficult to prove a negative**

  → solution: larger computational resources and
  "Multiple-pose 2D Free Energy Perturbation calculations"



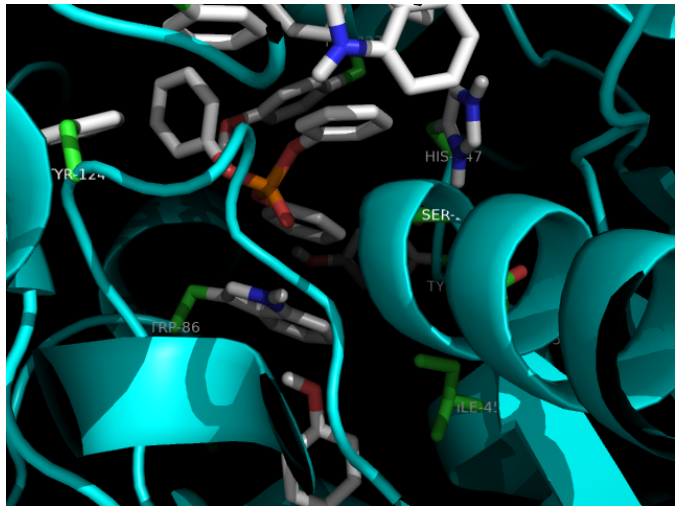
$$\Delta\Delta G_{\text{cor}} = -kT \ln\left(\frac{\exp\left(-\beta\Delta\Delta G_{\text{pose1}}\right) + \exp\left(-\beta\Delta\Delta G_{\text{pose2}}\right)}{2}\right)$$

Select the **most unfavorable substitution**, considering the most favorable of equivalent analogs (in terms of FEP direction or ring position due to rotation)

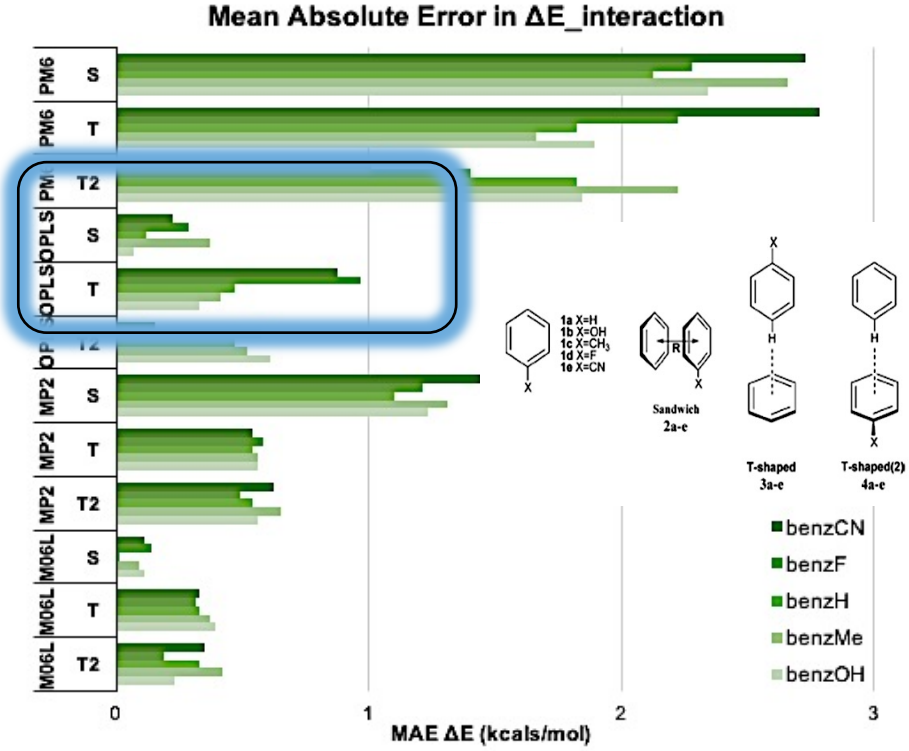
Average energetically-equivalent poses that are too different to be sampled:

Transforming MC/FEP to toxicant redesign

- ***Is our classical approach accurate enough, particularly to describe $\Pi-\Pi$ interactions between aromatic OPs and AChE residues***

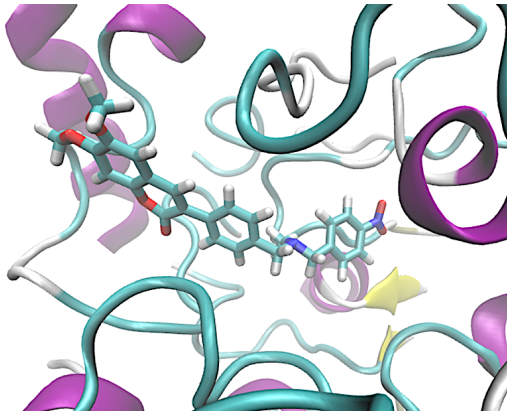


Aromatic OPs are stabilized in AChE active site primarily via $\Pi-\Pi$ S and T interactions. Our OPLS-AA/CM1A approach is reasonably accurate in describing these interactions.

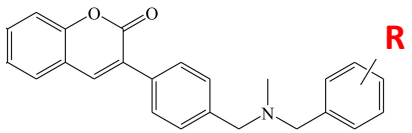


Benchmark: CCSD(T)/aug-cc-pVTZ

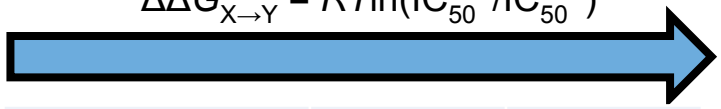
Method validation: AChE

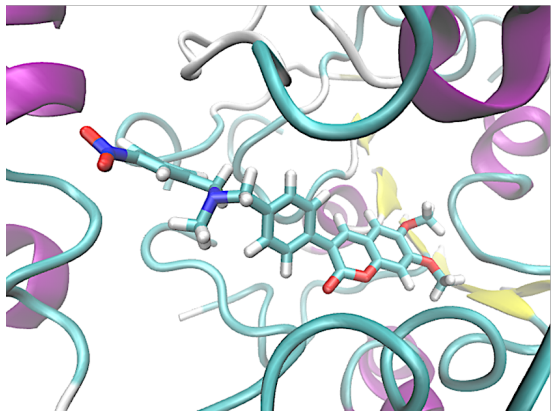


Perturbing position of substituents on inhibitor scaffold:

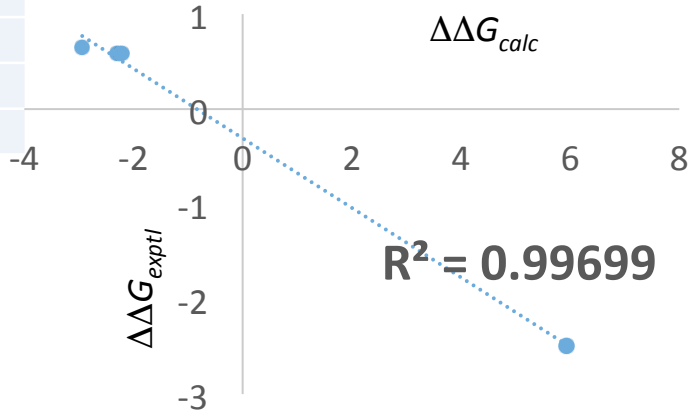


$\Delta\Delta G_{X\rightarrow Y} = RT\ln(IC_{50}{}^{X}/IC_{50}{}^{Y})$



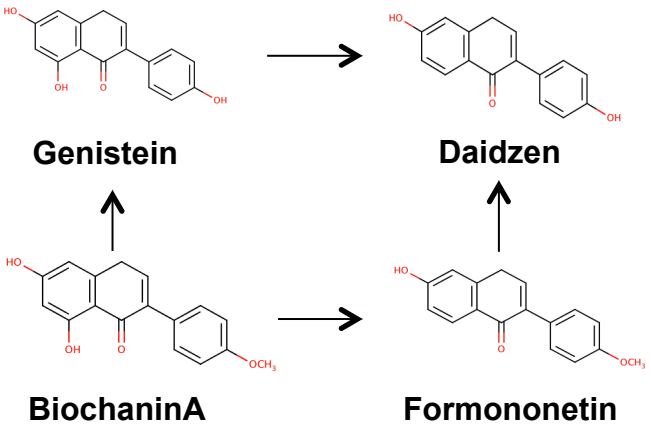


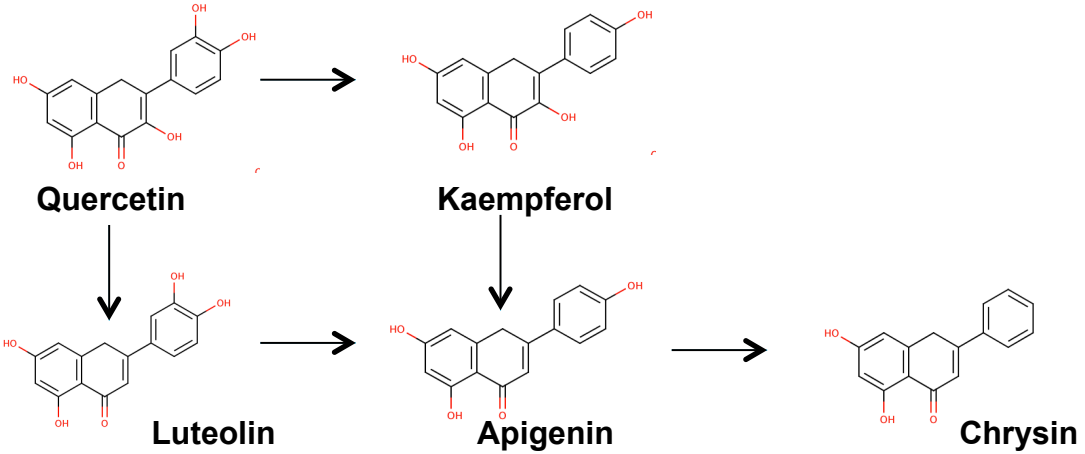
	$\Delta\Delta G_{calc}$	$\Delta\Delta G_{exptl}$
o-NO2→*m*-NO2	-2.3	0.58
m-NO2→*p*-NO2	5.9	-2.5
o-NO2→*o*-CH3	-2.2	0.59
m-NO2→*m*-CH3	-2.9	0.65



The accuracy in determining relative binding affinity using our MC/FEP protocol with OPLS-AA/CM1A/TIP4P force field (R^2 > 0.99) greatly exceeds that of docking software force fields (R^2 ~ 0.1).

Method validation: ERα

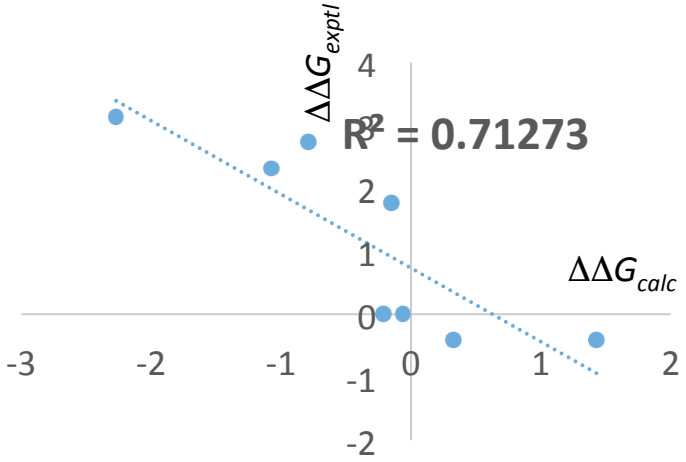




Analog	IC_{50} (M)
Genistein	$5x10^{-8}$
Daidzein	$1x10^{-7}$
BiochaninA	$1x10^{-5}$
Formononetin	$1x10^{-5}$

Analog	IC_{50} (M)
Quercetin	$5x10^{-5}$
Kaempferol	$5x10^{-5}$
Luteolin	$5x10^{-7}$
Apigenin	$1x10^{-6}$
Chrysin	$5x10^{-8}$

Method validation: ERα

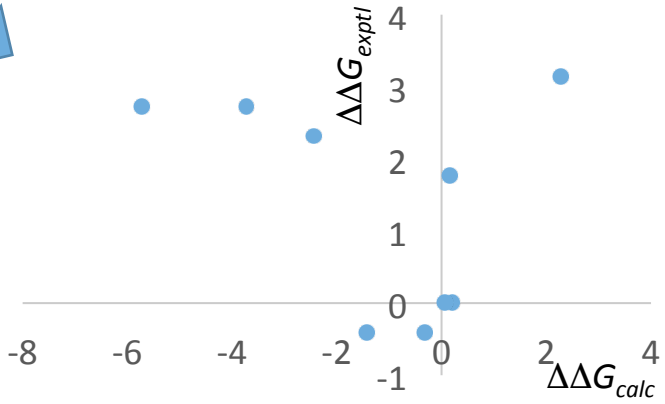


MC/FEP details:

- OPLS-AA/CM1A/TIP4P force field

- 14-20 windows (FEP steps)
- 5 M configurations of solvent equilibration
- 10 M configurations full system equilibration
- 20 M configurations of averaging

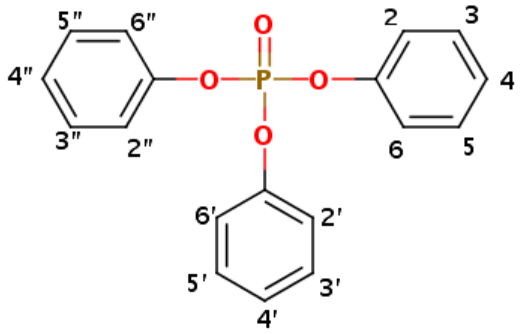
Without multiple poses: no correlation

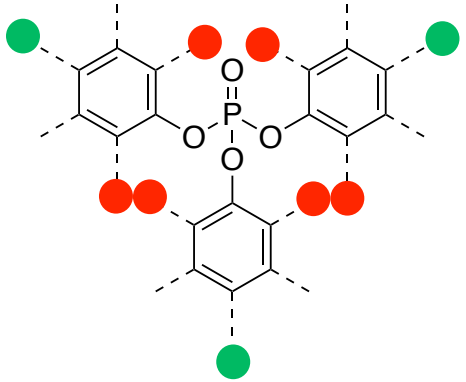


Chlorine and methyl scans

Chlorine Scan, Cl → H ΔΔG (kcal/mol)			
	Ring 1	Ring 2	Ring 3
C2	**-0.8**	**-4.2**	**-6.3**
C3	-5.6	-3.0	3.0
C4	-7.4	-3.0	2.6
C5	-0.1	-1.7	2.1
C6	**-5.2**	**-8.5**	**-7.1**

Methyl Scan, CH_3 → H ΔΔG (kcal/mol)			
	Ring 1	Ring 2	Ring 3
C2	-2.1	-1.5	-8.6
C3	-0.8	-5.4	-5.9
C4	**-4.1**	**-3.7**	**-8.6**
C5	**-6.6**	**-4.7**	**-8.7**
C6	2.5	-3.2	-3.7



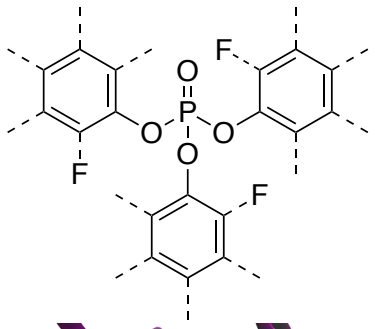


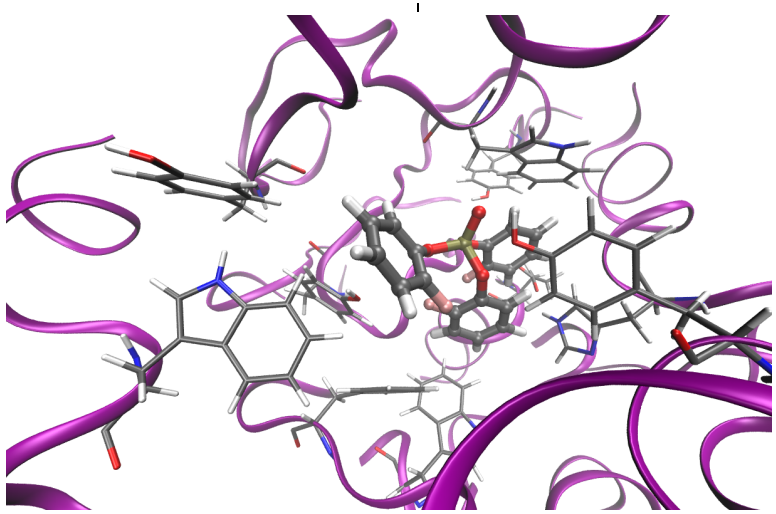
Electron-donating group diminishes binding affinity

Electron-withdrawing group diminishes binding affin

3H → 3F

$\Delta\Delta G_{bind}$ = +21.5 kcal/mol



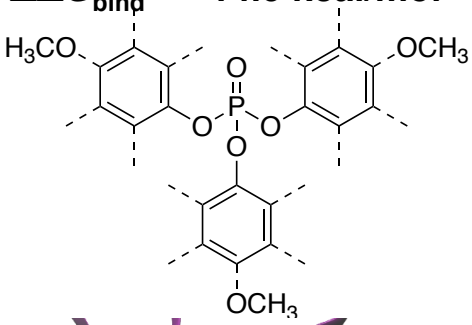


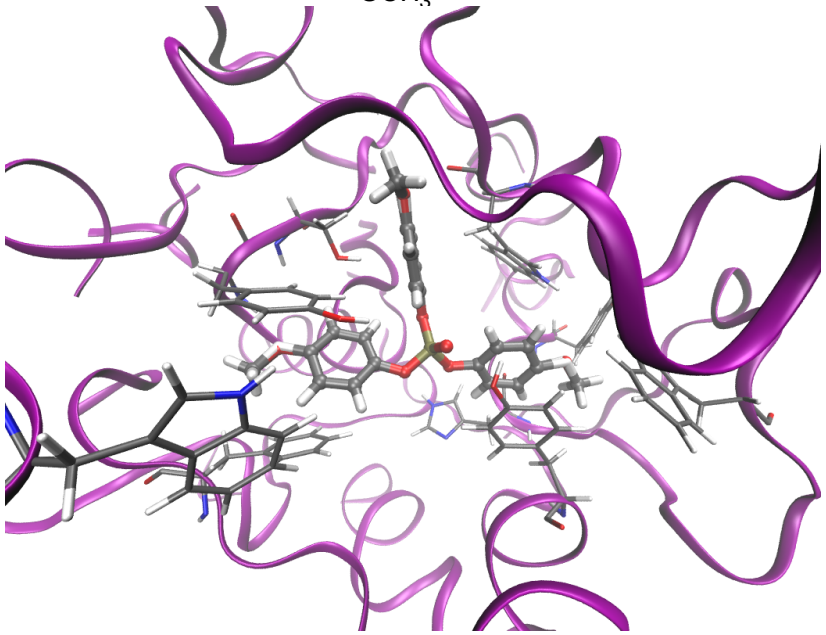
F is metabolically stable on ortho position and enhances flame-retardant function in the gas

3H → 3 –OCH_3

CH_3 → CH_2CH_3 → -OCH_3 (*p*)

$\Delta\Delta G_{bind}$ = +14.3 kcal/mol

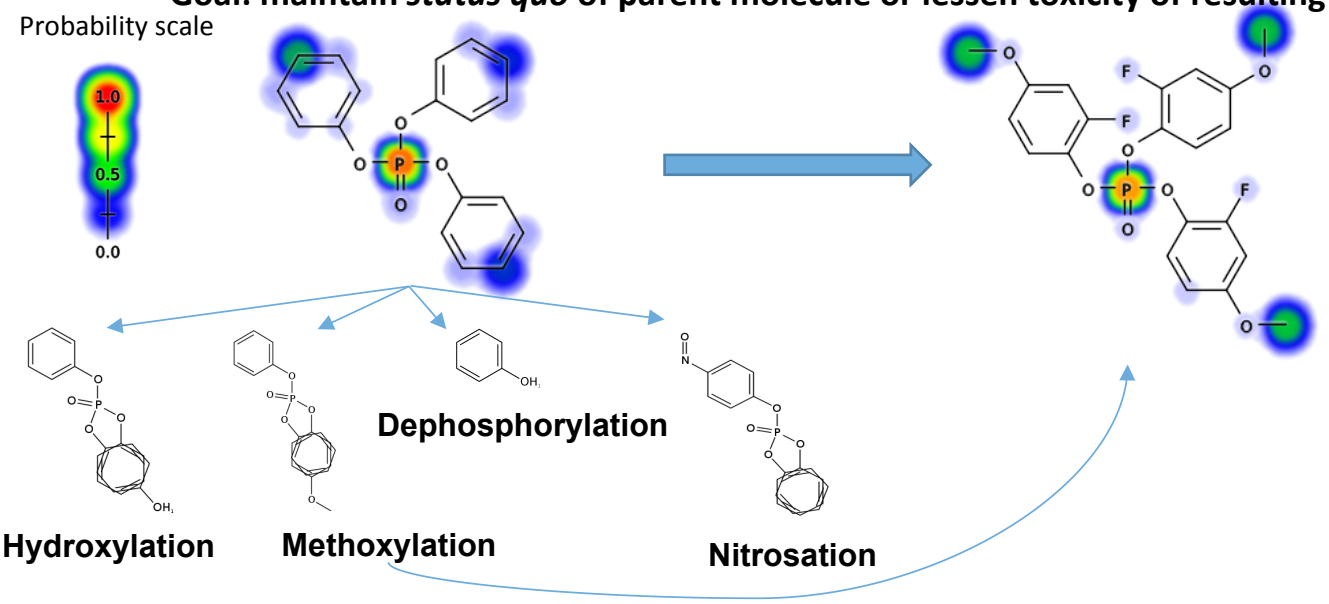




Can we benefit from combined effect of methoxy and X substitutions?

Metabolism

- Estimated using a consensus model: **XenoSite** (Phase I, 9 CYP isoforms, machine learning methods, structure-based) + **MetaPrint2D** (Phase I and II, fingerprinting- ligand based, developed using Accelrys Metabolite database)
- **Goal: maintain *status quo* of parent molecule or lessen toxicity of resulting metabolites**



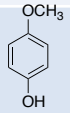
Detoxification propensity estimated with log $P_{o/w}$:

Met.	TPP	-3F	3 –OCH_3
D	1.7	1.8	1.5
H	4.8	5.2	-
M	4.9	5.4	-
N	5.2	5.6	-
Parent	5.1	5.5	4.6

Zaretzki, J., Matlock, M., & Swamidass, S. J. (2013). XenoSite: Accurately predicting CYP-mediated sites of metabolism with neural networks. Journal of chemical information and modeling, 53(12), 3373-3383.

Metabolism: TPP vs. metabolites

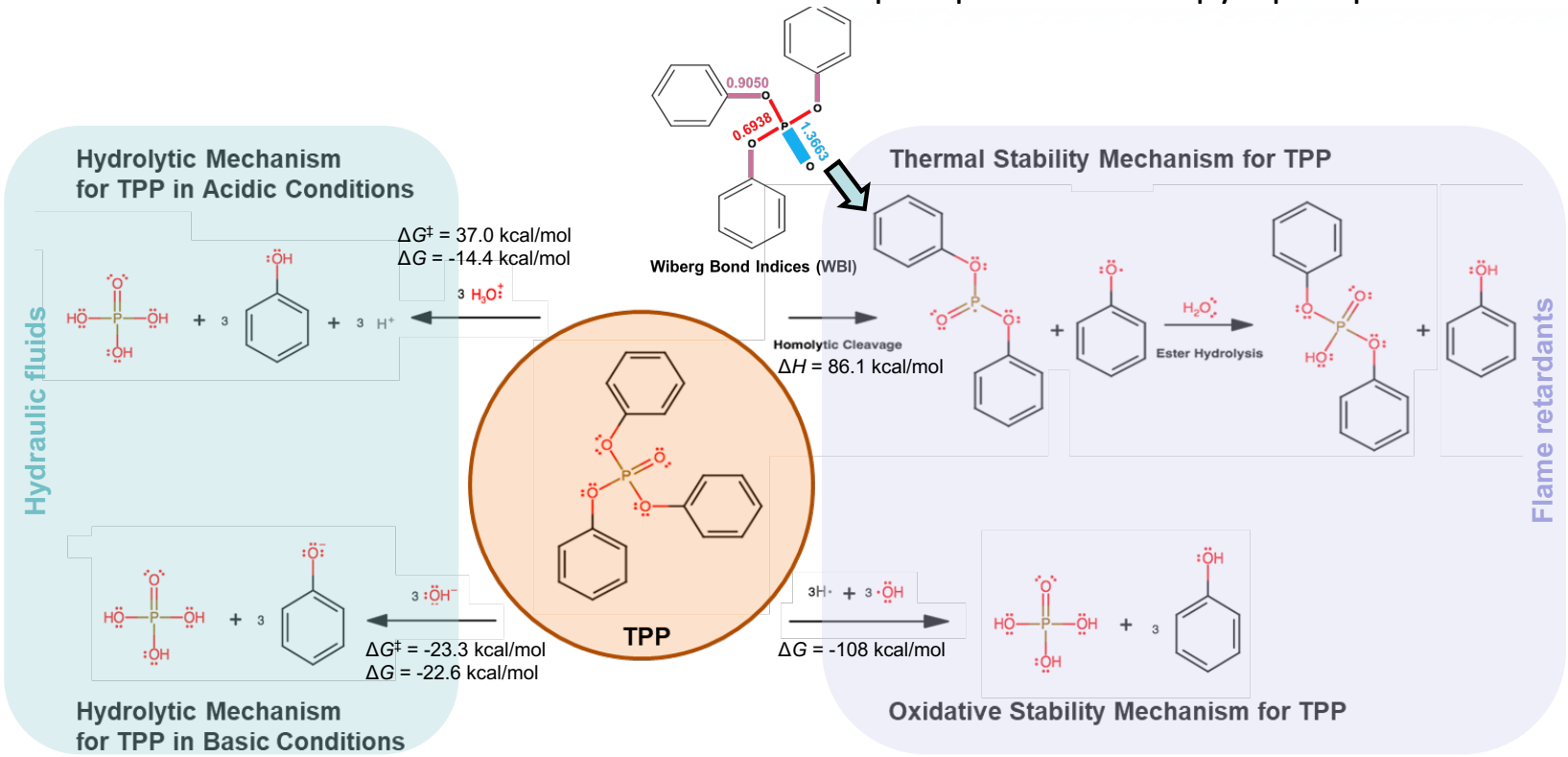
- Confirming that TPP is the form that inhibits AChE: ***no potent inhibitors among metabolites***

	Rel. binding affinity (kcal/mol)	Rel. population
TPP	**-**	-
DPP	+1.6	~7%
MPP	+4.4	~0.1%
Phenol	+5.1	~0.02%
	+4.3	~0.07%
	+4.3	~0.07%

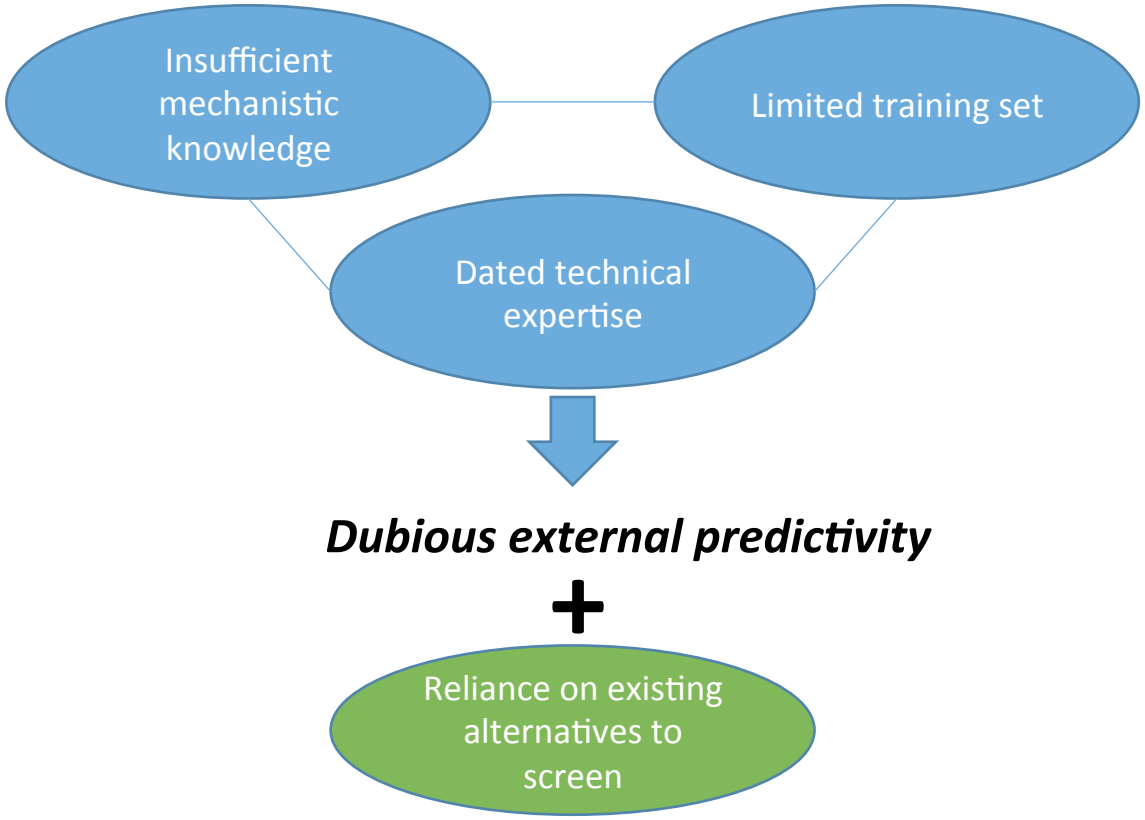
Metabolites of the two proposed analogs

Considering flame-retarding (FR) function

- X substitution increases flame retarding properties in the gas phase – additive effect to OP FR function: oxidative/thermal decomposition into phosphoric acid → pyrophosphoric acid

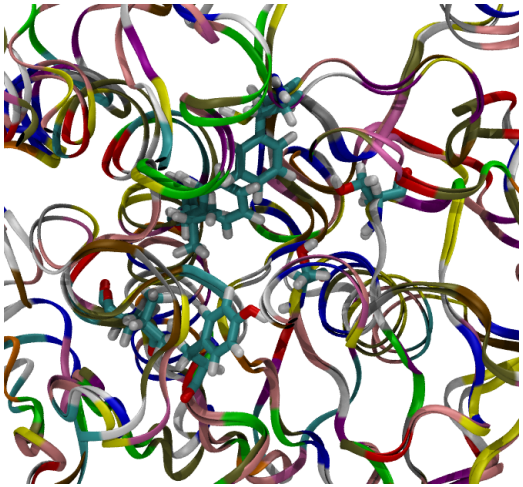


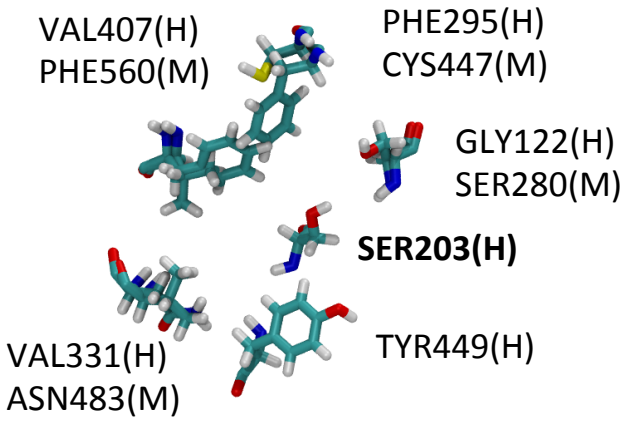
Does this approach alleviate the deficiencies of incumbent predictive modeling approaches?



Ongoing work and next steps

- Assessing TPP analogs (and relevant metabolites) against Era
- Consider QM modeling of SER phosphorylation in AChE
- *In vitro* testing against given targets; *in vivo* testing for aquatic toxicity (final stage for designed analogs)
- Synthetic feasibility and cost (e.g. fluorination vs chlorination)
- **Parallel project: OP pesticides (Malathion, Parathion)**





Acknowledgements

Group

CURRENT KRG MEMBERS

Eric Corcoran
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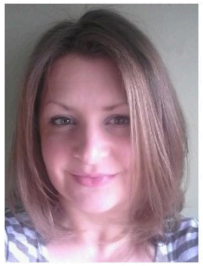
Vanessa Vargas
Graduate student



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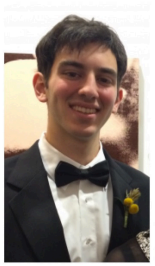
Postdoctoral researcher



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Helia Imany-Shakibai
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Facilities

http://kostal.columbian.gwu.edu/

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