Better Health, Brighter Future

Bigfoot, the Loch Ness Monster, and Halogen Bonds: Separating Rumors from Reality in Molecular Design

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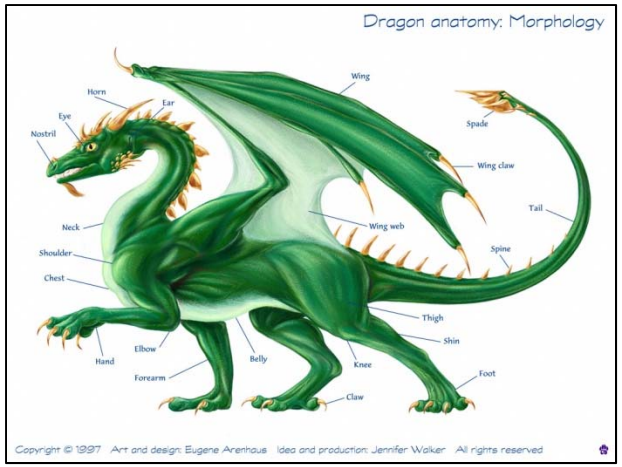
Cryptozoology

- The study of cryptids – “hidden animals”
  - Creatures believed to exist but for which there is no definitive evidence

- People believe because:
  - It’s hard to prove something doesn’t exist
  - There’s lots of anecdotal evidence
  - Believing has a nostalgic/romantic/fun quality

Medieval cryptid



Cryptid Interactions* in Drug Discovery

*Interactions that are *rumored* to exist

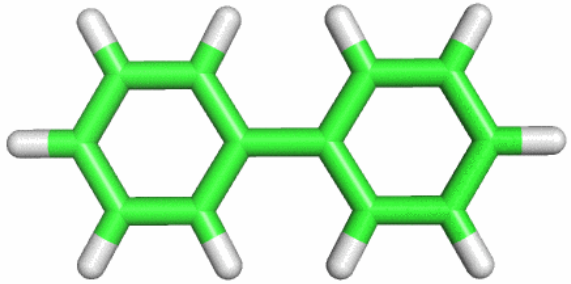
Why We Have Cryptid Interactions #1

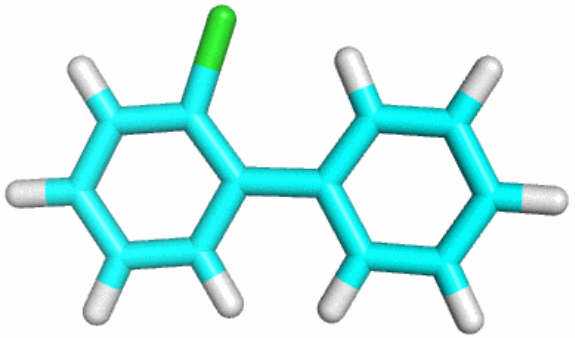
- Just because two atoms are next to each other in a crystal structure doesn't mean they're making a strong, favorable interaction
  - Sometimes the observed interaction is the least worst conformation with favorable interactions compensating for neutral/repulsive interactions
  - Take a close look at protein structures for some great examples
- Sometimes the electron density does not support the published structure (remember, crystal structures are really models)
  - See: Warren et al., *Drug Discov Today*, 2012,17(23-24):1270-8
- Sometimes the advertised interaction is only part of what's going on

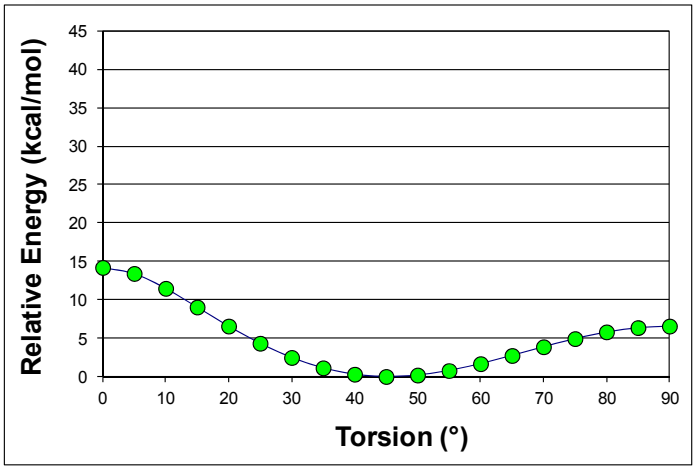
Why We Have Cryptid Interactions #2

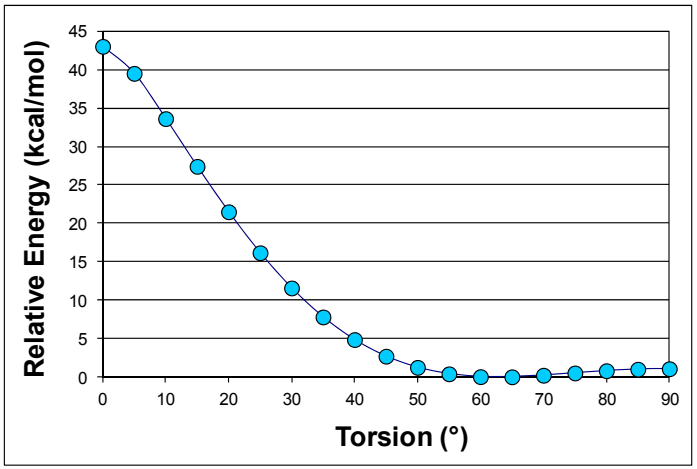
- The small atomic changes between molecule “pairs” often change multiple characteristics of a compound (electronics, conformation, etc.) Researchers often focus only on one effect of the change and ignore the others.

- Example:









Why We Have Cryptid Interactions #3

- Drug discovery runs on the experience of project team members:

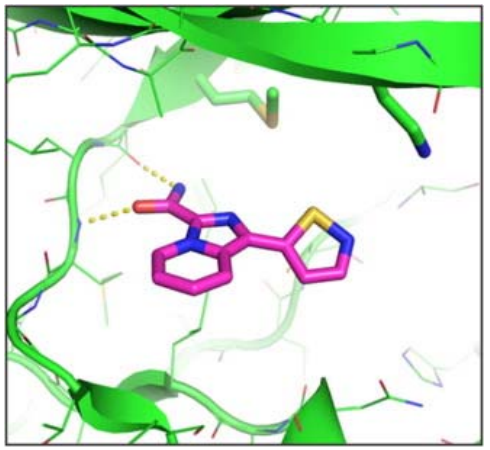
> "We should attach a *(functional group)* at that position because it increased potency 25-fold against *(protein name)* when I was on that project a couple of years ago."

- This is a great source of anecdotal evidence

Why We Have Cryptid Interactions #4

- The importance of particular molecular interactions are often overstated in the literature:

"The [30x] boost in potency [going from a pyridine to an isothiazole] could also result from a potential **sulfur-sulfur** interaction between Methionine-129 and the sulfur atom of the isothiazole ring."



Fragment-based drug discovery of potent and selective MKK3/6 inhibitors *BMCL*, 2016, 26(3): 1086-9

Mark Adams [a], Toshitake Kobayashi [c], J. David Lawson [b], Morihisa Saitoh [e], Kenichiro Shimokawa [e], Simone V. Bigi [a], Mark S. Hixon [c], Christopher R. Smith [a], Takayuki Tatamiya [e], Masayuki Goto [e], Joseph Russo [d], Charles E. Grimshaw [b], Steven Swann [a,*]

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[e] Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd, 26-1, Muraoka-higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

First Some Math…

Favorable Enthalpic Effects

- **H-bonding**
- **Ionic interactions**
- Other polar interactions
- **van der Waals**

HERE BE MONSTERS!

Favorable Entropic Effects

- **Hydrophobic "interactions"**
  i.e. waters from pocket to solvent

$$\Delta G_{bind} = \Delta H - T\Delta S = -RT \ln K_d$$

Unfavorable Enthalpic Effects

- Desolvation of polar groups*
- Conformational strain*
- Steric clashes
- Electrostatic repulsion

Unfavorable Entropic Effects

- Loss of conformational freedom*

***Occurs in both ligand and protein**

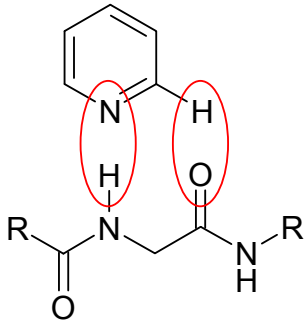
Weak Polar Interactions in Drug Design

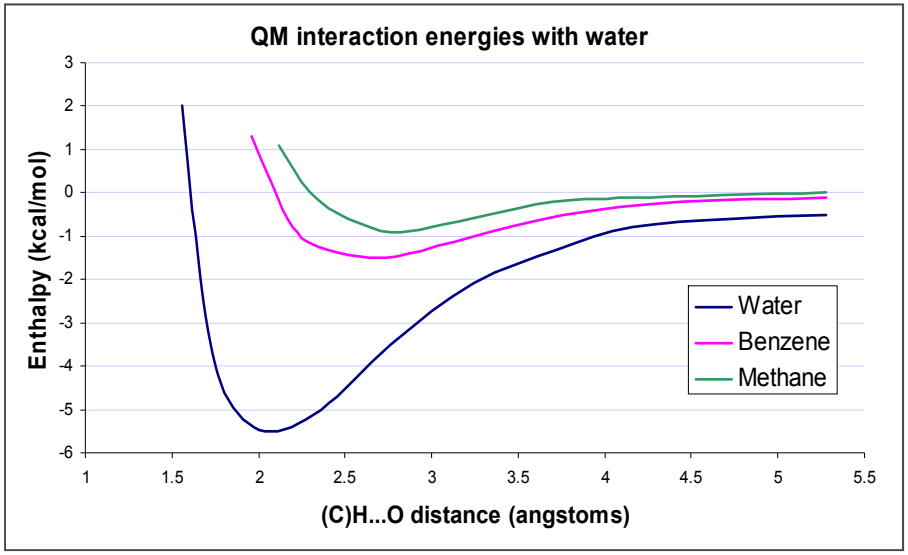
- Well documented/understood interactions:
  - Aryl CH pseudo H-bonds
  - Aryl ring – aryl ring interactions
  - Cation-π interactions

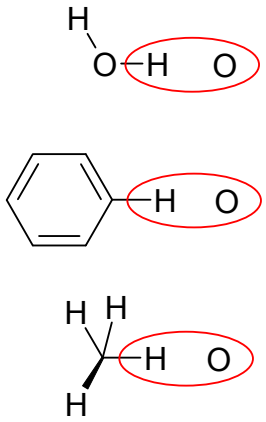
- Cryptid interactions?
  - Halogen bonds
  - Dunitz interactions
  - Sulfur-sulfur interactions
  - Sulfur as H-bond donor/acceptor
  - S-O:/S-N: interactions

Aryl CH Pseudo H-bonds

- Technically, not true hydrogen bonds: the proton is not exchangeable
  - The hydrogen is not shared between the 2 heavy atoms
- Distance-dependent, but less directional than true hydrogen bonds
- The distance between heavy atoms is larger and the penalty for close approach is harsher for C-H···O bonds relative to D-H···O bonds
  - D-H hydrogens are 'softer' than C-H (more polarizable)
- Not strong/directional enough to orient a group alone but will contribute to attraction if other groups drive orientation



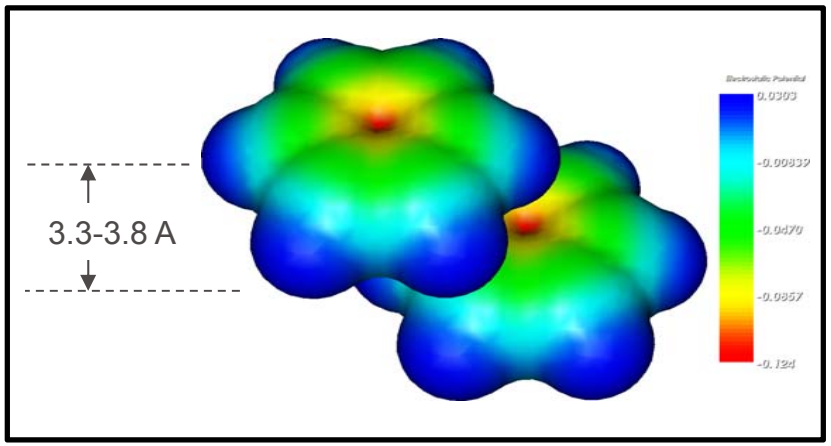


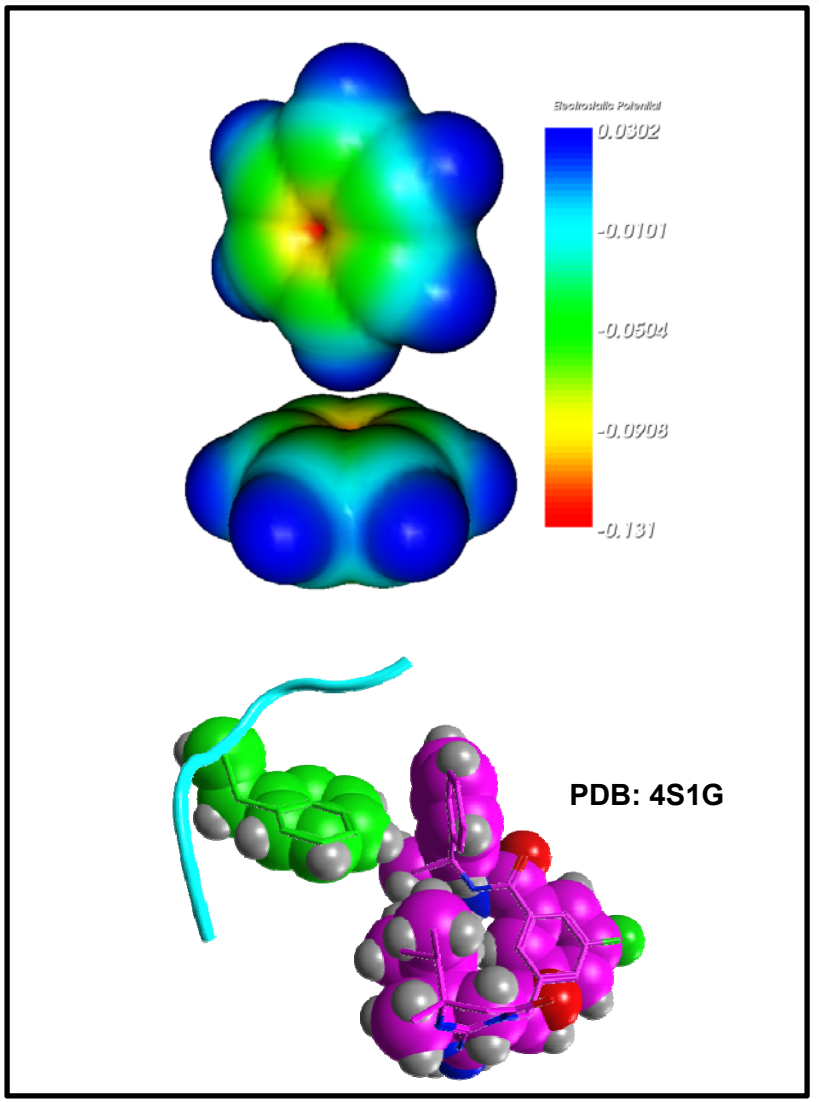


Bemis et al., *Proteins*, 2002, 49(4): 567-76

Aryl Ring – Aryl Ring Interactions

- Ring-Ring interactions are mainly driven by VdW interactions and entropic solvent displacement
- Keesom VdW, stronger than typical London dispersion
- Two main types of aryl ring – aryl ring interactions:
  - Edge-to-face interactions
  - Face-to-face interactions

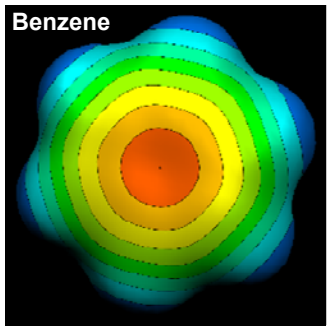


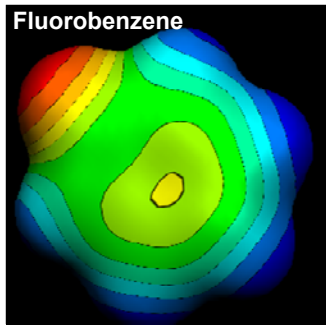


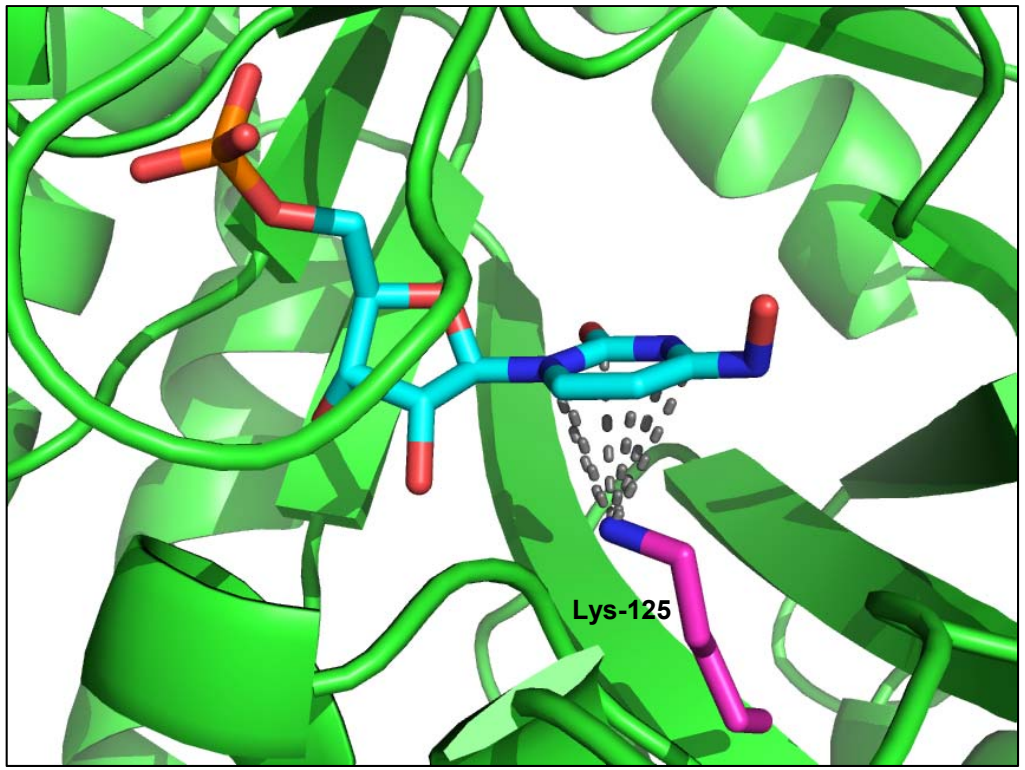
Cation-π Interactions

- Cation can be on protein (Lys, Arg, terminus) or ligand
- Often seen as part of protein structures (e.g. Arg/Trp interactions)

Aromatic ring electrostatics can be modulated:





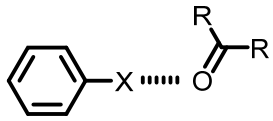


PDB: 4HIB

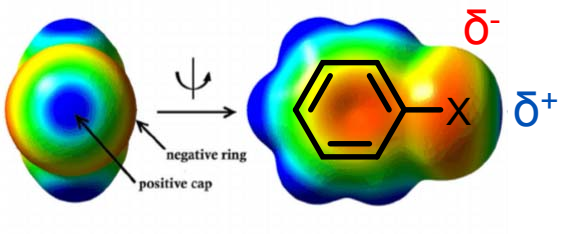
Warning: Entering Uncharted Territory…

The Elusive Halogen Bond

- Halogen bonds are **not** hydrogen bonds



- Halogen polarization is very anisotropic



Wilcken et al., *J. Med. Chem.* 2013, 56: 1363−1388

	Hydrogen Bonds	Halogen Bonds
Dipole-dipole interaction	Yes	Yes
Geometric dependency	Yes	Yes
"Shared" atom	Yes	No
Relative strength	Much stronger	Much weaker

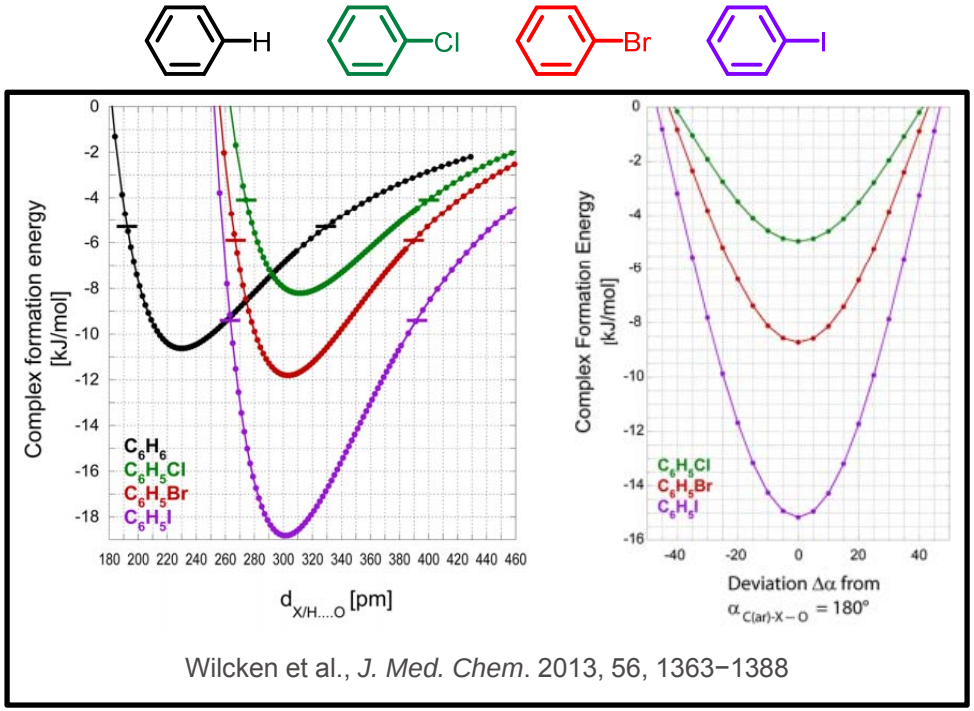
- The p_z-orbital participates in formation of the covalent σ-bond, leaving the orbital depopulated – this partially exposes the positive nuclear charge opposite the bond (the σ-hole)
- The σ-hole electron deficiency is compensated by an electron rich belt around the halogen
- Strength increases with the size of the halogen (because the electrons are more polarizable): Cl < Br < I (not fluorines)

Charge surface from: Yang et al., *J. Mol. Model* 2015, 21: 138

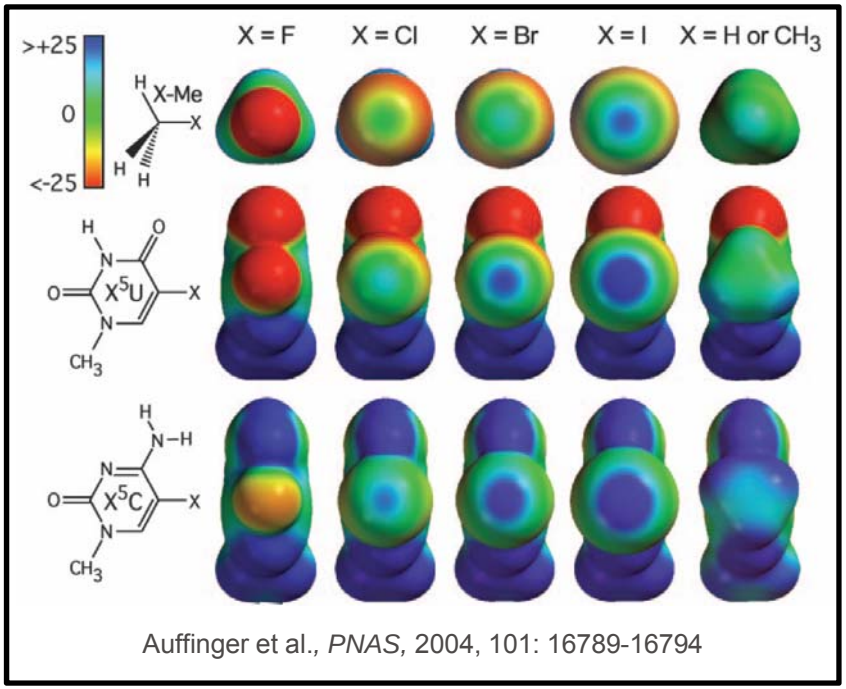
Halogen Bonds in Molecular Design

σ-hole strength can be modulated

(Note: F is always electronegative)

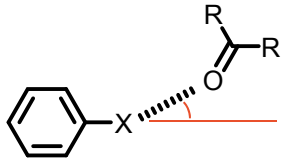


Wilcken et al., *J. Med. Chem*. 2013, 56, 1363−1388



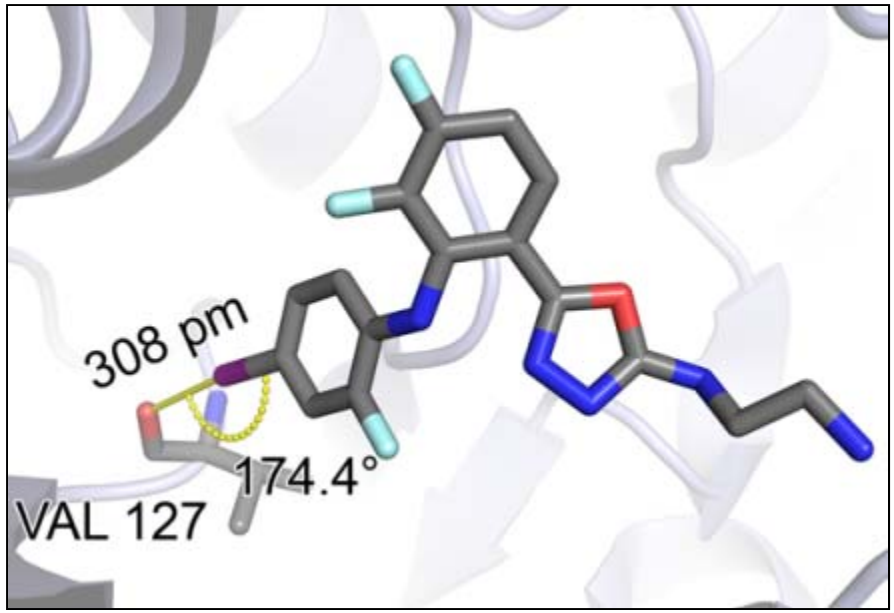
Auffinger et al., *PNAS*, 2004, 101: 16789-16794

Halogen bonds have a stringent geometric requirement

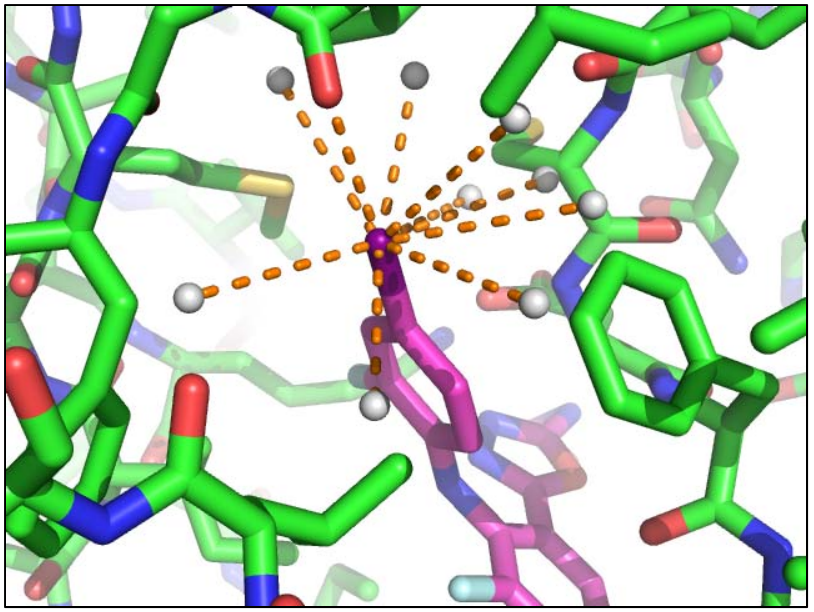


The σ-Hole isn't Everything

- In addition to the X•••O interaction, the permanent dipole of the electro-negative halogen "waist" interacts with induced electropositive dipoles of surrounding hydrogens:
  - Debye Forces (between permanent/induced dipoles) are stronger that London Dispersion Forces (between induced/induced dipoles)



MEK1 crystal structure 3EQB as shown in Wilcken et al., *J. Med. Chem*. 2013, 56, 1363−1388



A closer look reveals that >90% of the iodine surface interacts with the surrounding hydrogen atoms

Hydrogen/Halogen vs. Oxygen/Halogen Interactions

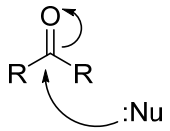
- 10,924 liganded structures from PDB (curated ligands)
  - Protonate the structures using GBSA
  - Minimize the protons

Halogen	#	Atoms: 4.5A	Hydrogens (% of atoms)	Oxygens (% of atoms)	C--X—O ≥ 170° (% of O found)	Nitrogens (% of atoms)	Carbons (% of atoms)
I	103	2,297	1,319 (57%)	160 (7.0%)	20 (12.5%)	131 (5.7%)	669 (29%)
Br	284	6,280	3,692 (59%)	314 (5.0%)	7 (2.2%)	305 (4.9%)	1,927 (31%)
Cl	1,472	25,678	14,155 (55%)	1,741 (6.8%)	34 (2.0%)	1,258 (4.9%)	8,319 (32%)
F	1,074	43,514	25,058 (57%)	2,817 (6.4%)	28 (1.0%)	2,265 (5.1%)	13,617 (31%)

- Bigger halogens → more surface to interact with hydrogens
  → more solvent displacement

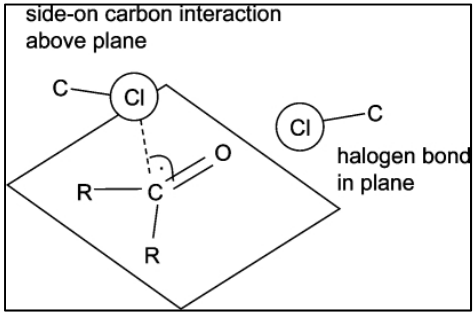
Dunitz Interactions

- The carbon atom in a carbonyl is slightly electropositive

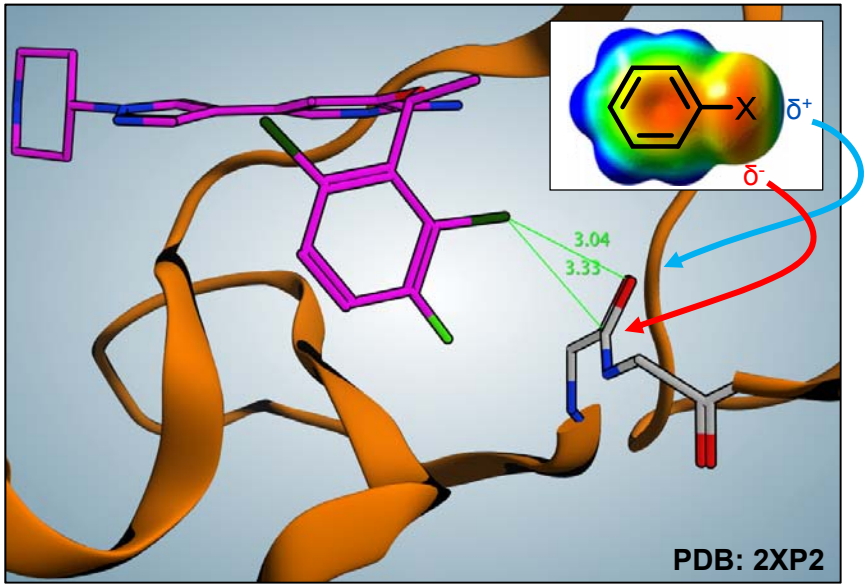


- Interactions with the carbonyl carbon are known as Bürgi-Dunitz interactions or (usually) Dunitz interactions
  - The partner is weakly electronegative and has the correct sterics to approach the carbonyl (F and Cl are the usual suspects – sulfurs work too)
  - These are relatively weak dipole/dipole interactions

- Interaction with a chlorine can form a multipole interaction:

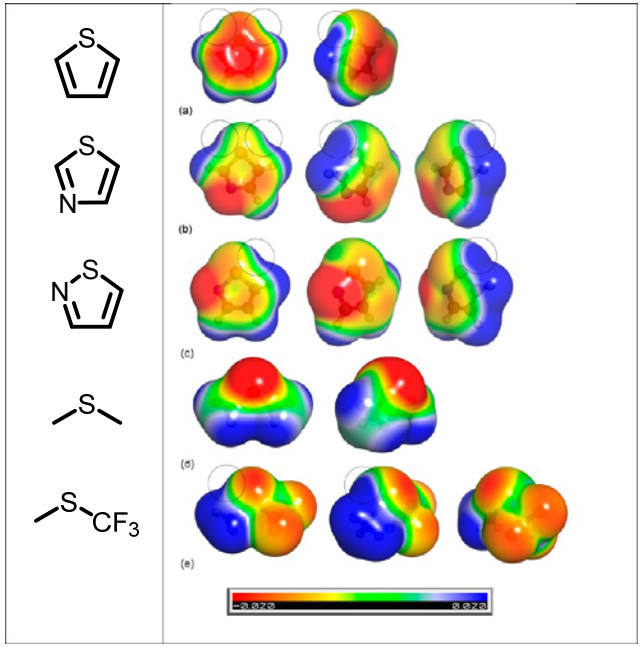


Bissantz et al., *J Med Chem.* 2010 Jul 22; 53(14): 5061–5084.

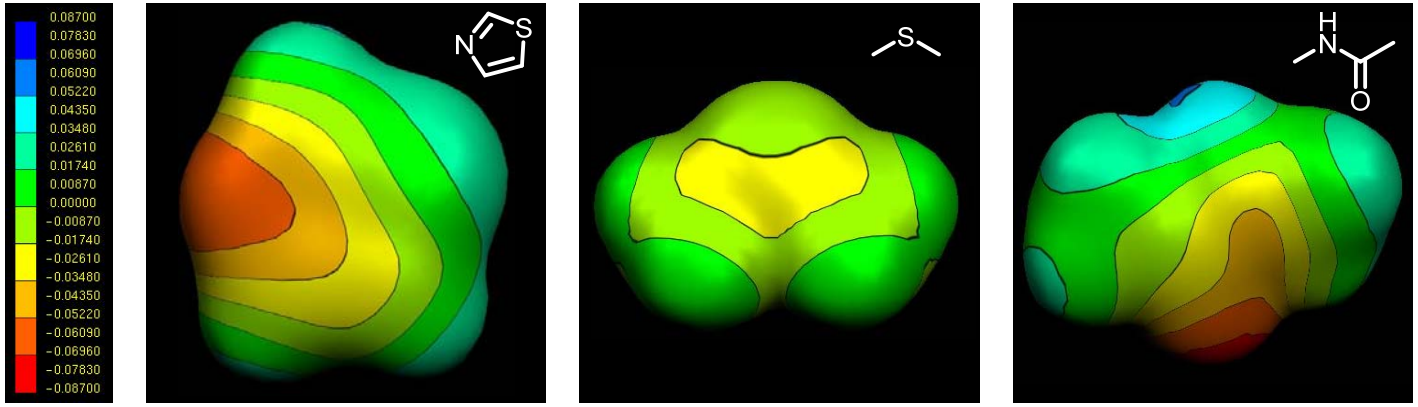


Sulfur-Sulfur Interactions

- Usually occur between an aromatic sulfur in the ligand and a Met or Cys residue in the protein
  - Thiols and alkyl thioethers not usually used for medchem
  - Thiones and diaryl thioethers are uncommon
  - The sulfur of sulfones and sulfonamides is not accessible.
- Sulfurs are mostly big and lipophilic
  - Methionine sulfurs are slightly δ-
  - Aromatic sulfurs are slightly δ+ and have a σ-hole like halogens
  - Diffuse electrons → polarizable and malleable shape
- Like when adding halogens, more that one thing changes when a sulfur is introduced (conformation, electronics, etc.)



Beno et al., J. Med. Chem. 2015, 58: 4383−4438

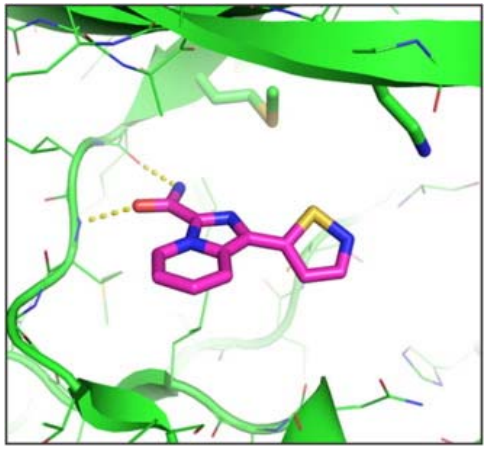


Replotted with electrostatic scale normalized to amide

Sulfur-Sulfur Interactions

- From the MKK3/6 example shown earlier:

> "In this model, the isothiazole is within an acceptable distance for a favorable interaction with Lysine-82. Additionally, the sulfur likely maintains the planarity of the system, as well as the preferred conformation of the ring. The boost in potency could also result from a potential **sulfur-sulfur** interaction between Methionine-129 and the sulfur atom of the isothiazole ring."



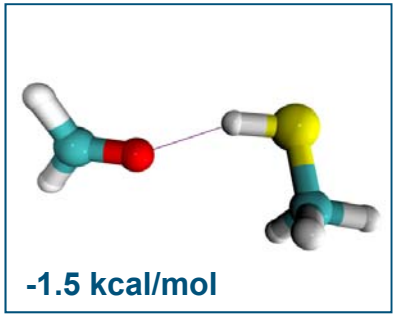
  - Changing 4-pyridine to isothiazole yields a 30x potency boost
  - S•••S interaction is plausible:
    - The sigma hole of aryl sulfur is roughly oriented towards Met sulfur
    - The distance is reasonable within error (this is a model, not a crystal structure)
  - There are several other factors that are contributing to the potency increase
    - Higher quality H-bond to the Lys (electronics and geometry)
    - Less ligand strain

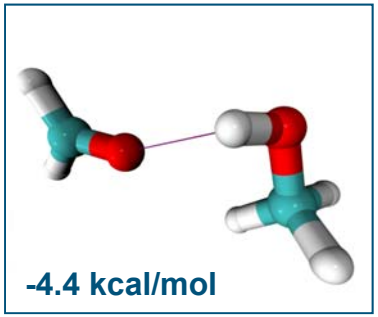
Adams et al., *BMCL*, 2016, 26(3): 1086-9

Sulfurs and H-Bonds

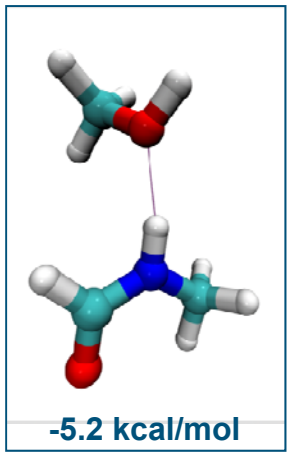
- Sulfurs are not great H-bond donors (cysteine donating to ligand)
  - About ~1/3rd the strength of normal H-bonds
- Sulfurs are poor H-bond acceptors
  - Probably won't make up the desolvation penalty
  - Watch out for the σ-hole!

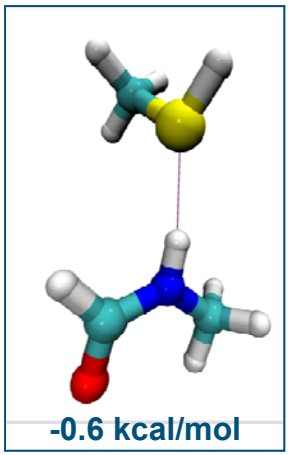
Sulfur as Donor

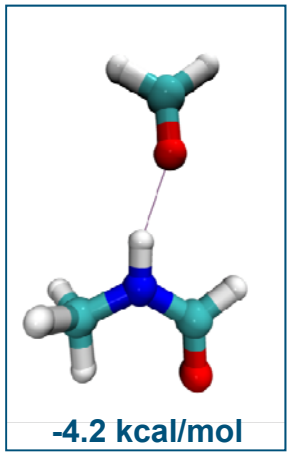


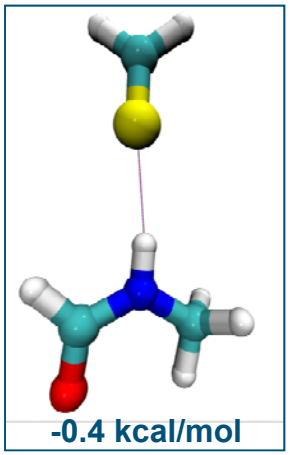


Sulfur as Acceptor



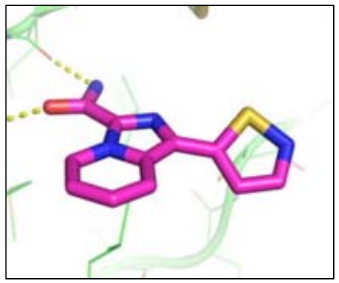






Sulfur-Oxygen & Sulfur-Nitrogen Interactions

- S•••O: and S•••N: interactions appear to be important for intramolecular interactions
  - Lower energies involved in influencing a few dihedrals
  - Scaffolding enables easy access to favorable geometries



- No overwhelming evidence for impactful intermolecular interactions
  - Higher energy needed to be relevant for protein-ligand interactions
  - Beno et al., J. Med. Chem. 2015, 58: 4383−4438

- "Theoretical and crystallographic data investigations of noncovalent S•••O interactions" (Junming et al., *Structural Chemistry,* 2011, 22(4): 757-63)

  - 50,000 X-ray crystal structures w/ Met and 3.0 Å or better resolution
    - ~14% of structures contained ≥1 Met-S•••O=C contacts meeting geometry/distance cutoff
    - Total of 12,830 contacts (0.25/structure average)
    - Caveat: only about half of them are making putative σ-hole interactions

  - Control: 10,812 human X-ray crystal structures w/ Met and 2.2 Å or better resolution
    - ~95% of structures contained ≥1 Met-NH•••O=C hydrogen bonds
    - Total of 98,653 contacts (9.1/structure average)

The Cryptid Scorecard

- Well documented/understood interactions:
  - Aryl CH pseudo H-bonds
  - Aryl ring – aryl ring interactions
  - Cation-π interactions

- Cryptid interactions?
  - Halogen bonds
  - Dunitz interactions
  - Sulfur-sulfur interactions
  - Sulfur as H-bond donor
  - Sulfur as H-bond acceptor
  - S-O:/S-N: interactions

Intramolecular interactions okay

EL CHUPACABRA

The Bottom Line

- Most (but not all) of these “cryptid” interactions are real, favorable intermolecular interactions
  - Individual atoms are polarized – different faces have different electrostatics
  - All of these interactions have many subtleties that impact their effect on potency
- These interactions by themselves won’t bring you huge gains in potency
  - None of these interactions are as strong as H-bonds
  - Sometimes large potency gains are observed due to the direct enthalpic interaction **plus** other effects (e.g. entropic effects, electronic changes, conformational preference changes)
- **Focus molecular design on shape complementarity, H-bonds, and low strain**
  - A single hydrogen bond can offset the entropy loss from ligand ordering and will orient a molecule due to the angles, properties, and strength of the bond
- **These interactions are most beneficial once a ligand has already been ordered, as entropy has been already paid**
  - Considerer them as secondary interactions once the majority of the ligand ordering entropy has been paid

The Bottom Line

Just because something isn't a cryptid doesn't mean it's not cool

Thanks!

Andy Jennings

Tony Ivetac

Steve Wilkens