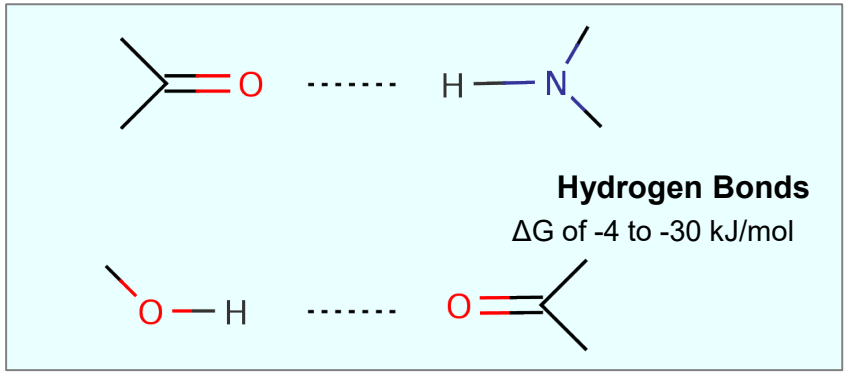
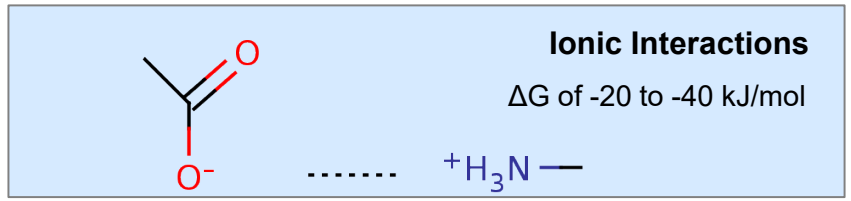
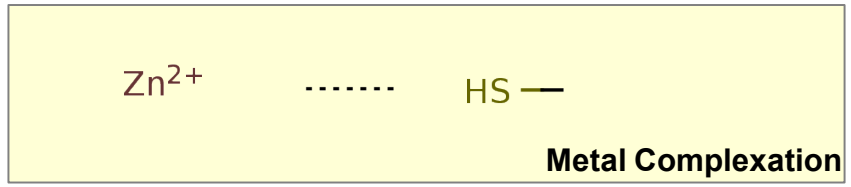
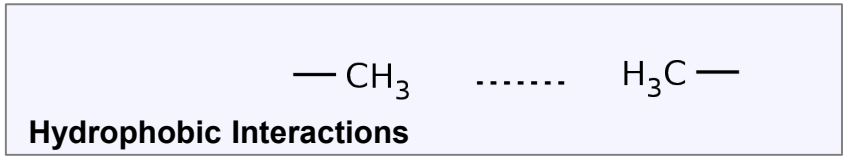
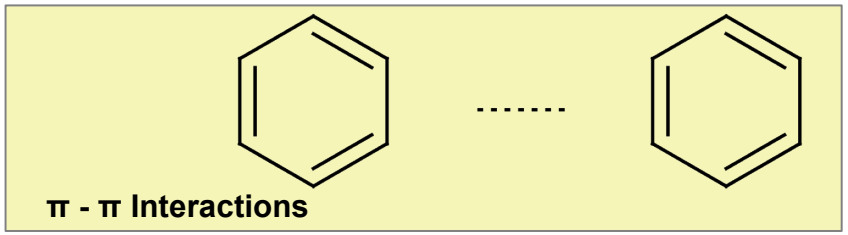
# Take a New Look In the Old Toolbox:

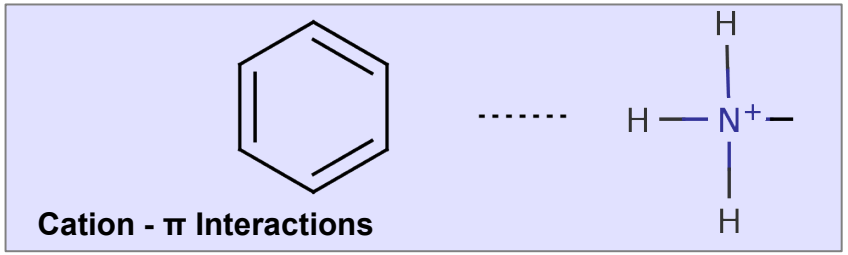


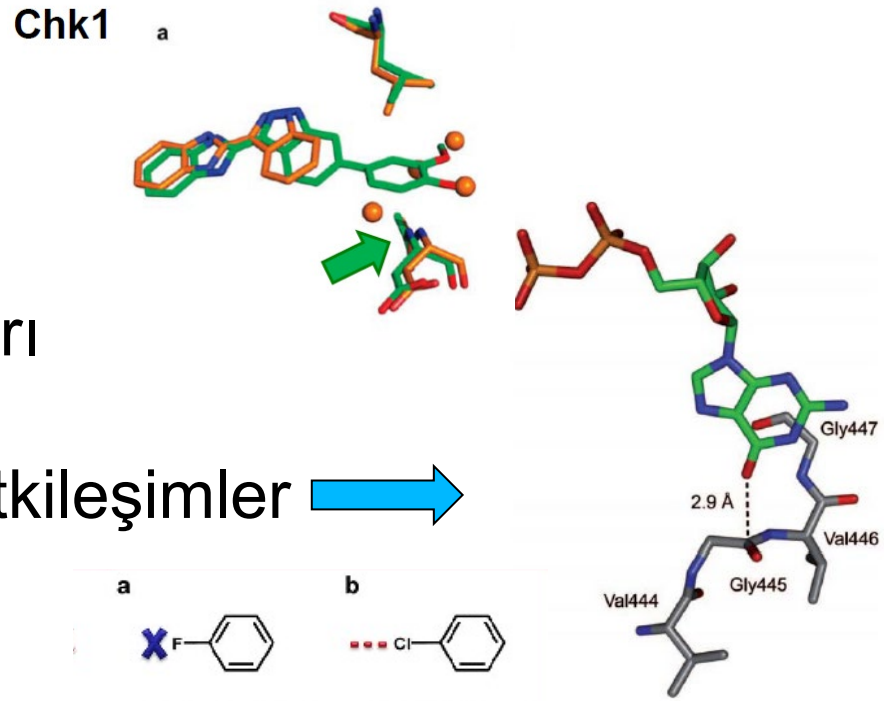



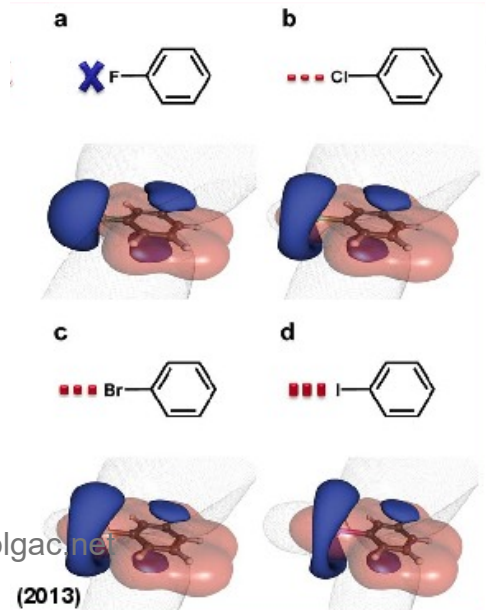



Wermuth, The practice of medicinal chemistry, Academic Press, 2008

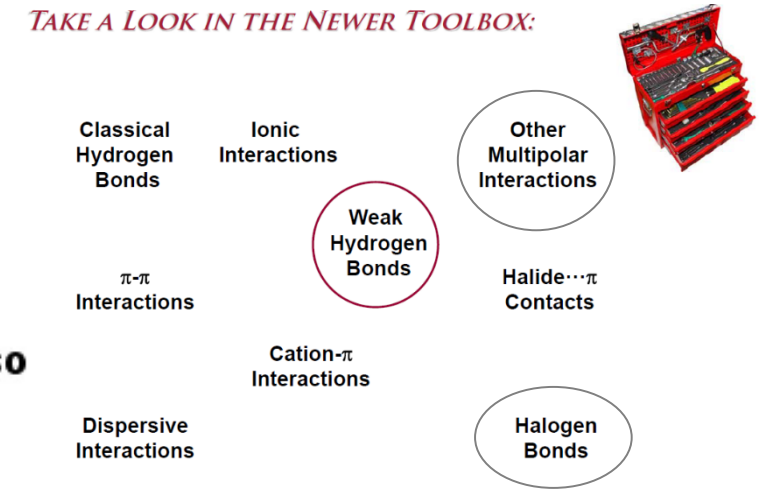
# Neglected Interactions in Molecular Recognition

- Zayıf Hidrojen Bağları
- Dikey Çoklu Polar Etkileşimler
- **Halojen Bağları**



# Weak Hydrogen Bonds




Increased attention on weak hydrogen bonds

➜ double edge sword:

recognize previously unnoticed interactions, but also increases the risk of overinterpretation!

Weak hydrogen bonds often contribute to protein-ligand binding in a subtly directional fashion

➜ important to understand that they are, at the very least, not repulsive!

Aromatic rings can be acceptors of hydrogen bonds

➜ benzene - ammonia interaction: ~ 2.2 kcal/mol,
in comparison: benzene – methane interaction: ~ 1.5 kcal/mol

➜ NH vector is oriented perpendicularly to the ring plane at the benzene ring center

Aromatic ring can only be a "reserve" acceptor for a strong donor!

➜ Tyr, Phe, and in particular Trp side chains interact more frequently with polarized CH groups as donors!

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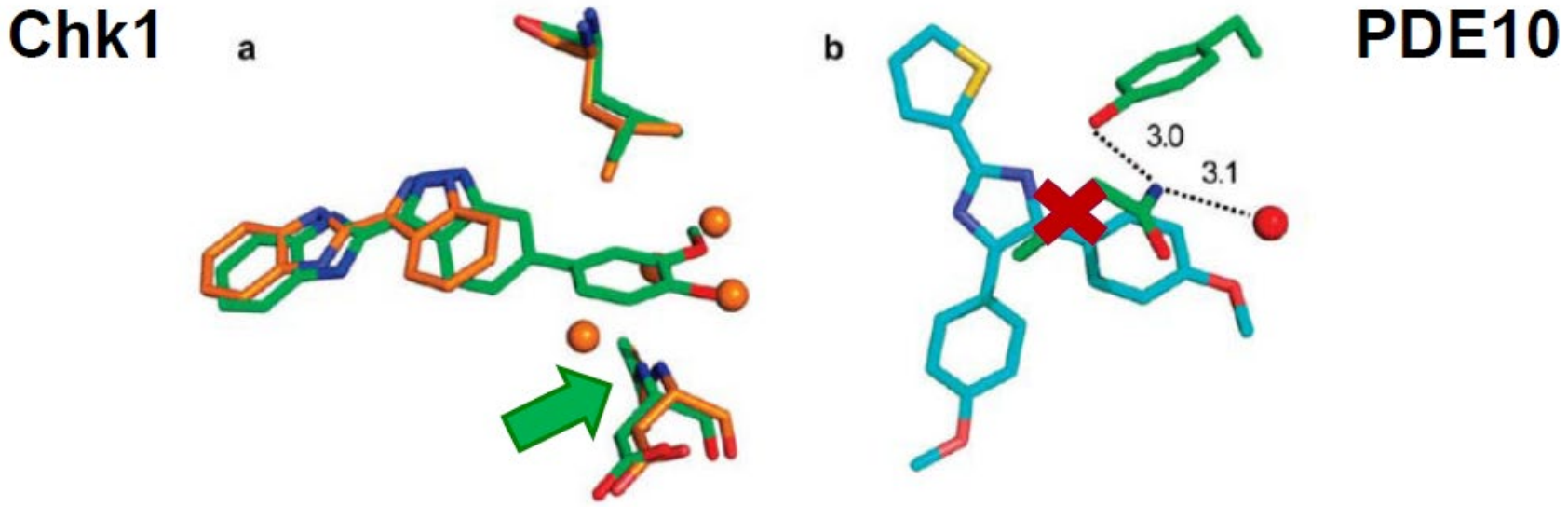
Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

# Weak Hydrogen Bonds




Structures referred to as NH - π interactions in the literature.

(a) **Chk1 kinase ligands.**

The orange structure has a Ki of 8.5 μM (PDB code 2c31)
The green structure has a Ki of 0.026 μM (PDB code 2c3k)

The additional phenyl ring displaces several water molecules, one of which was coordinated to the backbone NH below. The shortest NH – Phenyls distance in the green structure is 3.4 A°.

(b) **A PDE10 complex structure**
where in reality the glutamine side chain forms two classical hydrogen bonds to water and a tyrosine.

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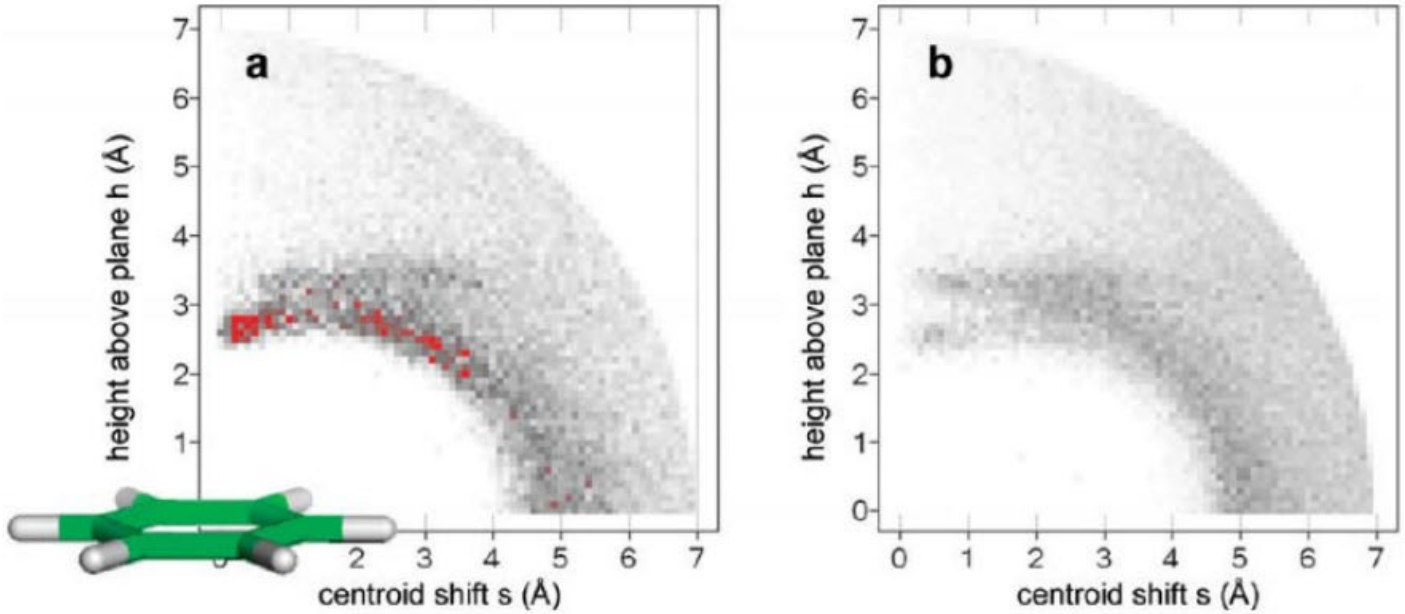
Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

# Weak Hydrogen Bonds

**Hydrogen atoms in X-CH units polarized by neighboring heteroatoms (X = O, N) have a clear preference for interactions above the ring plane, whereas NH and OH groups rarely undergo π hydrogen bonds. (NH···π stronger than CH···π, but gain is overcompensated by a higher desolvation cost!)**




Radial distribution of hydrogen atoms around a phenyl ring (CSD Statistics):
Hydrogen bound to;
**(a)** $sp^2$ carbon flanked by one or two heteroatoms (N,O) **(b)** O or N.
The phenyl ring in (a) is drawn roughly to scale and should serve as an interpretation aid for the in plane (*s*) and above plane distances (*h*).
Darker gray corresbonds to higher density; peaks above a numerical values of 70 are colored red.

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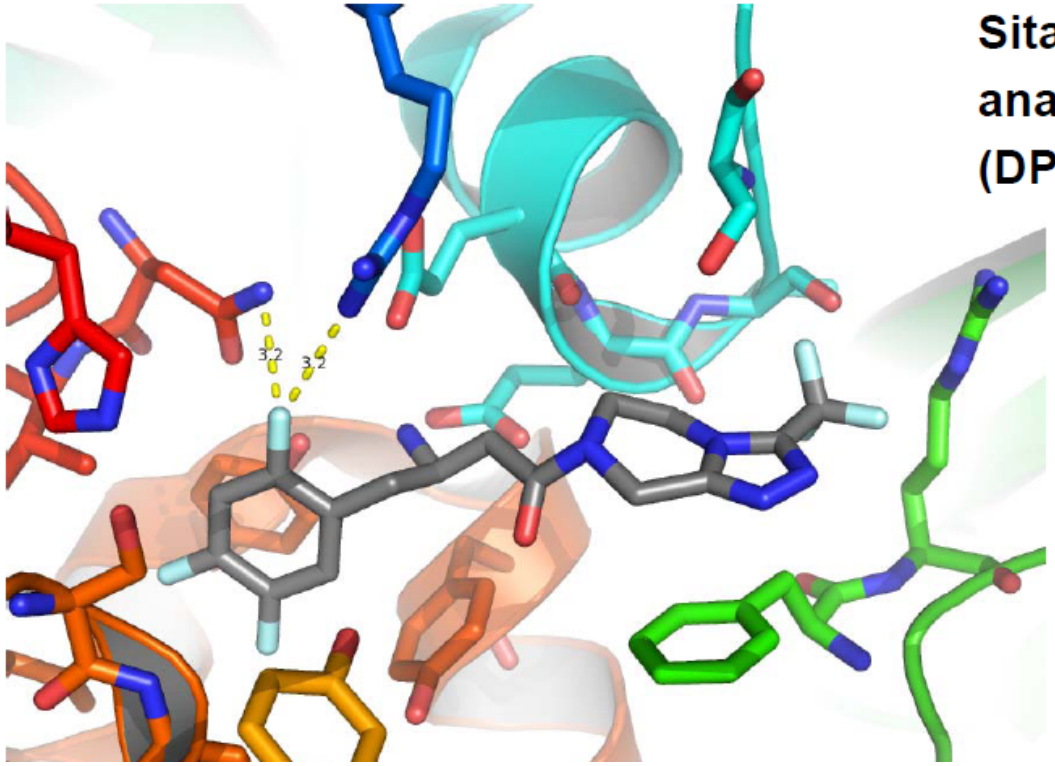
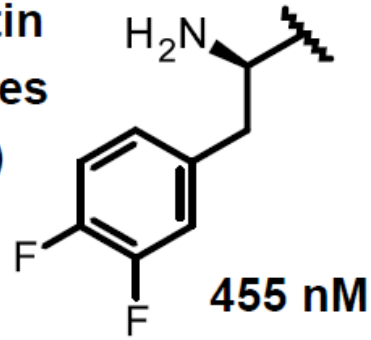
# Weak Hydrogen Bonds

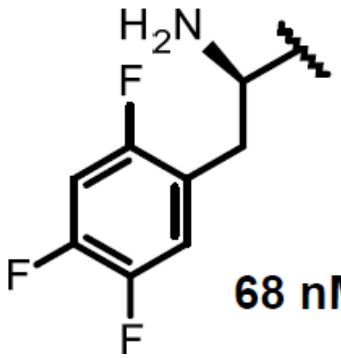
Interactions between C-F and polar hydrogen atoms H-X (where X=O, N) frequently occur in the PDB and CSD, even if such interactions cannot be classified as strong hydrogen bonds.




Sitagliptin analogues (DPP-IV)




455 nM




68 nM

7



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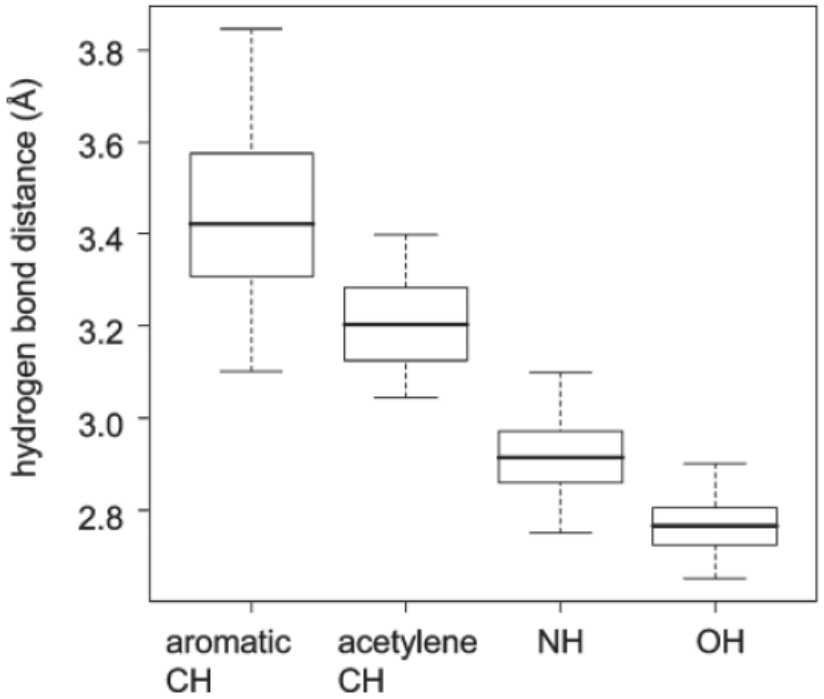
# Weak Hydrogen Bonds

**The weaker a hydrogen bond donor, the longer is the hydrogen bond distance and the broader is the distribution of the observed distances in crystallographic databases.**




**weaker interactions at larger distances**

**typically expected hydrogen bond distance**

Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

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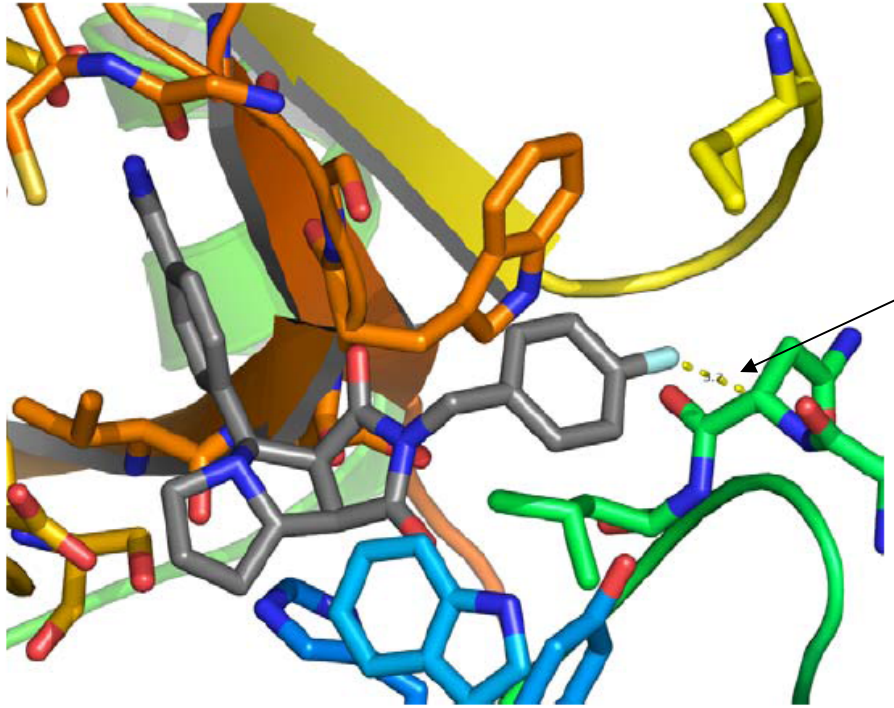
# Weak Hydrogen Bonds

**Cα-H unit of proteins is also a weak donor.**

**Calculations indicate that Cα-H···O=C interactions are about one-half the strength of an N-H···O=C hydrogen bond.**



**thrombine inhibitors**

**→ Cα-H···F-C interaction (3.2 Å)**

**1OYT: thrombine inhibitors**

Olsen et al., Angew. Chem. Int. Ed. 42, 2507 (2003)

Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

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# Weak Hydrogen Bonds

Investigations of model systems led to the conclusion that aliphatic-aromatic and aromatic-aromatic edge-to-face contacts provide similar levels of stabilization.

➜ 60-fold lower $IC_{50}$ value by introducing a carbonyl group adjacent to the nitrogen atom of the morpholine ring (Rivaroxaban)

➜ polarization of the ring $CH_2$ on top of Trp (C-Trp dist.: 3.5 Å)

➜ increase $CH_2 \cdots \pi$ interaction and preorganize the perpendicular orientation to adjacent phenyl group

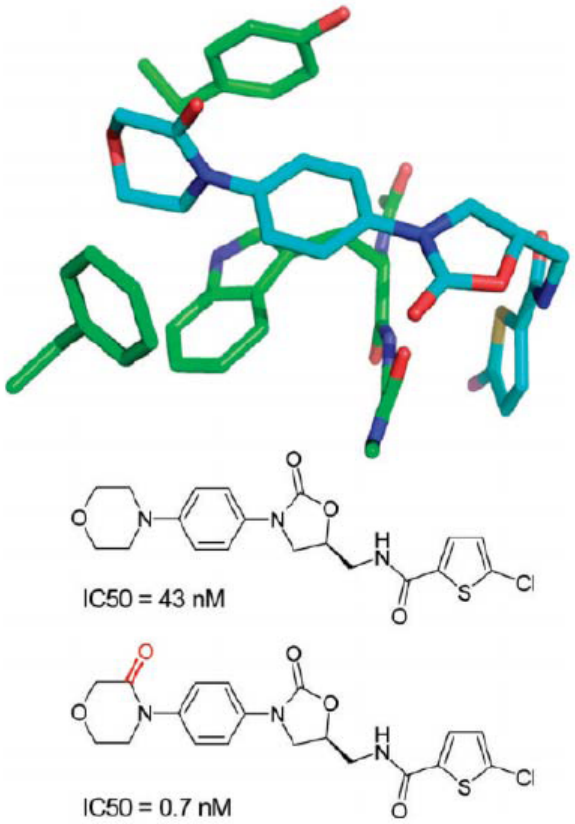


**Figure 17.** Structure and $IC_{50}$ values[168] of factor Xa inhibitors (PDB code 2w26, distances in Å).

Roehrig *et al.* J. Med. Chem. 48, 5900 (2005)
Bissantz *et al.* J. Med. Chem. 53, 5061 (2010)

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# Weak Hydrogen Bonds

Beneficial effect of acidifying CH groups on top of an edge-to-face oriented phenyl ring:

p38 MAP kinase inhibitor:

- Insertion of oxygen between amide and methyl group

➜ methylhydroxamat: 200-fold increased affinity!

➜ CH-π, CH-Carbonyl interaction by significantly acidified C-H !

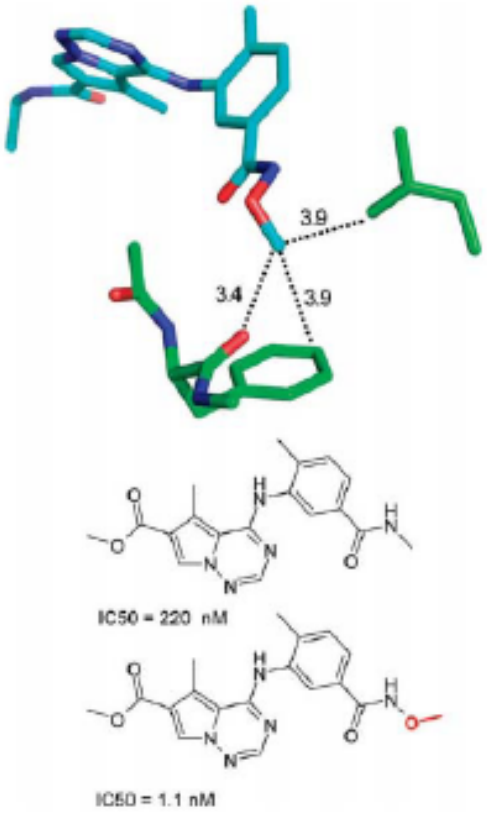


**Figure 18.** Effect of a methyl group on the $IC_{50}$ of a p38 MAP kinase inhibitor[198] (PDB code 2rg5).

Hynes *et al.*, J. Med. Chem. 51, 4 (2008)

Bissantz *et al.* J. Med. Chem. 53, 5061 (2010)

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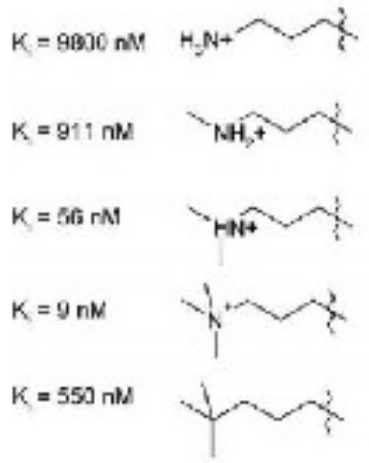
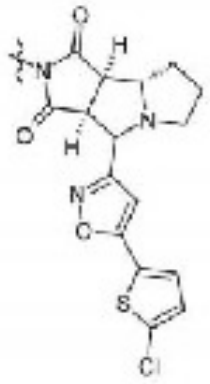
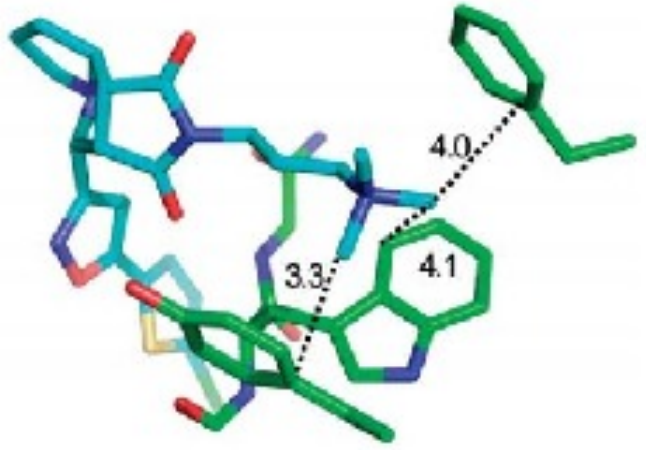
# Weak Hydrogen Bond = Cation Pi Interactions

**Alkylammonium ions and aromatic rings**
**➜ special class of alkyl-aryl interaction.**
**A (formally) positively charged nitrogen is a particularly strong electronegative substituent ➜ strongly attractive interaction!**




Salonen *et al.*, Angew. Chem., Int. Ed. 48, 811 (2009)
Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

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# Orthogonal Multipolar Interactions

- characterized by a close orthogonal contact between two dipolar functional groups
- completely orthogonal arrangement between two dipoles
  ➜ actual dipole contribution to interaction energy = 0
  ➜ higher order electrostatic and dispersion terms must be responsible for the attractiveness of the interaction!

The disappearance of the dipole term may turn a repulsive electrostatic interaction into an attractive one.

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# Orthogonal Multipolar Interactions

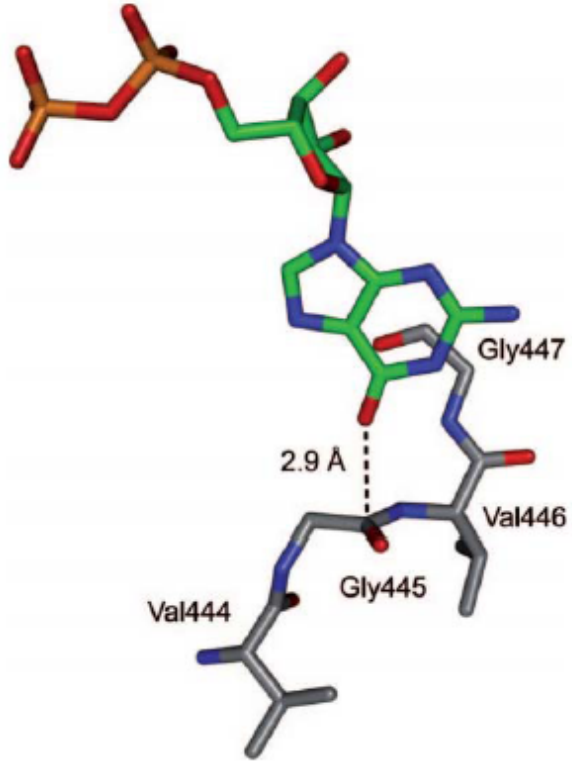


**Fig. 1.** Section of the X-ray crystal structure of the complex between adenylosuccinate synthetase and GDP (PDB ID code 1lny; 2.20-Å resolution, EC 6.3.4.4) showing an orthogonal dipolar C=O···C=O interaction (18). Color code: ligand skeleton, green; C, gray; O, red; N, blue; P, orange.

Iancu *et al.*, J. Biol. Chem. 277, 26779 (2002)

Fischer *et al.*, Proc. Natl. Acad. Sci. U.S.A. 105, 17293 (2008)

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# Orthogonal Multipolar Interactions

***Table 1:*** Overview of dipolar interactions in small molecule crystal structures as retrieved from the CSD (version 5.25, November 2003).[a]

| A–B | E–Y | $d_{vdW}$ [Å] | Search range [Å] | Number of structures | Number of occurrences | Number of occurrences within $d_{vdW}$ |
|---|---|---|---|---|---|---|
| C–F | C=O | 3.30 | 2.30–3.80 | 599 | 1028 | 243 |
| C–Cl | C=O | 3.65 | 2.65–4.15 | 1742 | 2968 | 816 |
| C–Br | C=O | 3.80 | 2.80–4.30 | 1105 | 1770 | 547 |
| C–I | C=O | 4.00 | 3.00–4.50 | 228 | 399 | 143 |
| C–OH | C=O | 3.25 | 2.25–3.75 | 587 | 775 | 144 |
| C=O | C=O | 3.25 | 2.25–3.75 | 16546 | >10000 | 1888 |
| $R_2C$=O | $R_2C$=O | 3.25 | 2.25–3.75 | 2614 | 4031 | 966 |
| $H_2O$ | C=O | 3.25 | 2.25–3.75 | 814 | 1122 | 48 |
| $H_2O$ | $R_2C$=O | 3.25 | 2.25–3.75 | 35 | 36 | 1 |
| $C_2O$ | C=O | 3.25 | 2.25–3.75 | 1093 | 1358 | 330 |
| $C_2O$ | $R_2C$=O | 3.25 | 2.25–3.75 | 197 | 223 | 48 |
| C–F | C–$NO_2$ | 2.95 | 1.95–3.45 | 38 | 51 | 5 |
| C–Cl | C–$NO_2$ | 3.30 | 2.30–3.80 | 154 | 198 | 9 |
| C–Br | C–$NO_2$ | 3.45 | 2.45–3.95 | 77 | 98 | 5 |
| C–I | C–$NO_2$ | 3.65 | 2.65–4.15 | 34 | 59 | 7 |
| C=O | CN | 3.25 | 2.25–3.75 | 443 | 626 | 130 |
| C–OH | CN | 3.25 | 2.25–3.75 | 27 | 35 | 1 |
| $C_2O$ | CN | 3.25 | 2.25–3.75 | 140 | 273 | 84 |
| $C_2S$ (C: $C_{sp^2}$) | CN | 3.70 | 2.70–4.20 | 30 | 57 | 16 |
| CN | CN | 3.35 | 2.35–3.85 | 2080 | 5912 | 448 |
| C–F | CN | 3.30 | 2.30–3.80 | 110 | 247 | 58 |
| C–Cl | CN | 3.65 | 2.65–4.15 | 180 | 404 | 97 |
| C–Br | CN | 3.80 | 2.80–4.30 | 100 | 227 | 66 |
| C–I | CN | 4.00 | 3.00–4.50 | 34 | 73 | 32 |
| C–F | R′-$CN_2$ | 3.30 | 2.30–3.80 | 19 | 28 | 7 |
| C–Cl | R′-$CN_2$ | 3.65 | 2.65–4.15 | 74 | 109 | 33 |
| C–Br | R′-$CN_2$ | 3.80 | 2.80–4.30 | 33 | 50 | 18 |
| C–I | R′-$CN_2$ | 4.00 | 3.00–4.50 | 10 | 12 | 9 |
| C=O | R′-$CN_2$ | 3.25 | 2.25–3.75 | 561 | 861 | 90 |
| $H_2O$ | R′-$CN_2$ | 3.25 | 2.25–3.75 | 190 | 258 | 17 |
| $C_2S$ | R′-$CN_2$ | 3.70 | 2.70–4.20 | 38 | 46 | 17 |
| $C_2O$ | R′-$CN_2$ | 3.25 | 2.25–3.75 | 68 | 96 | 18 |
| C–F | C–F | 3.30 | 2.30–3.80 | 1989 | >10000 | 2111 |
| C–F (C: $C_{sp^2}$) | C–F (C: $C_{sp^2}$) | 3.30 | 2.30–3.80 | 926 | >10000 | 2581 |

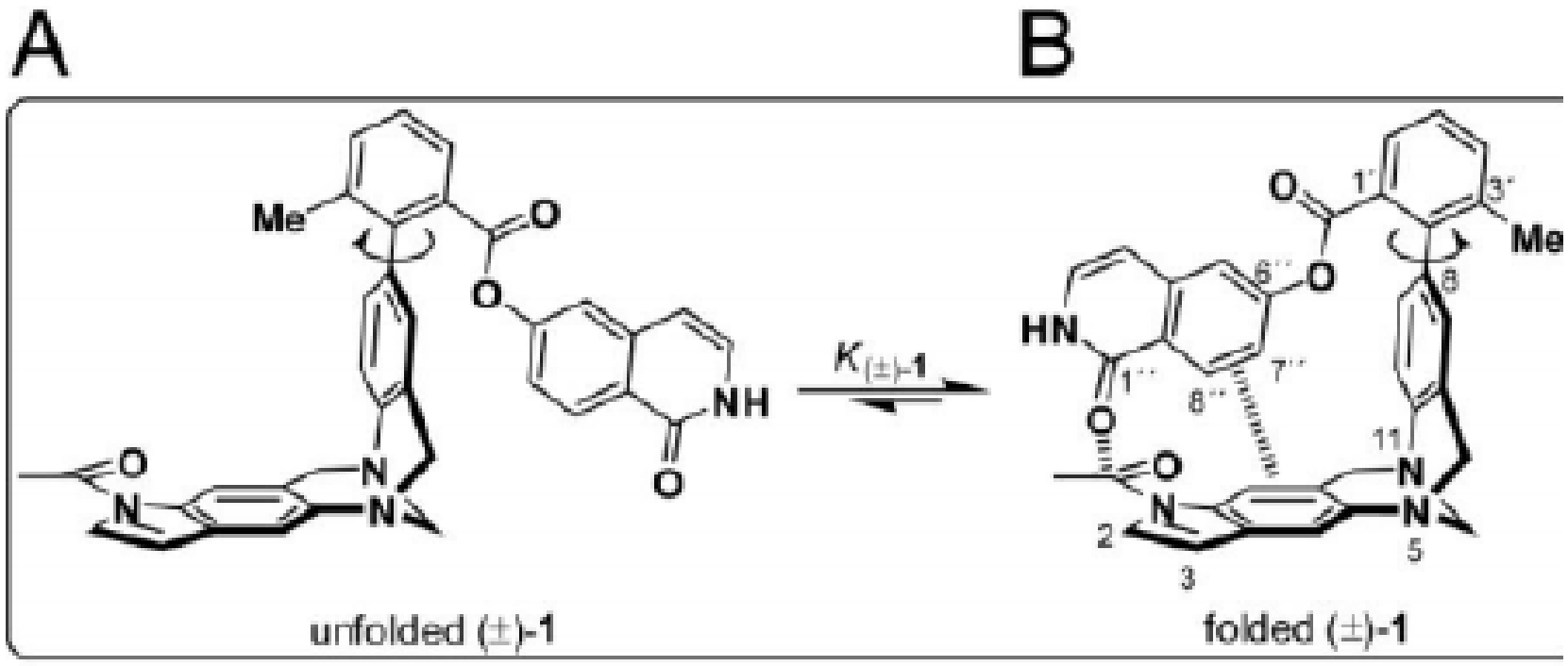
**15**



Paulini *et al.*, Angew. Chem. Int. Ed. 44, 1788 (2005)

# Orthogonal Multipolar Interactions

**Model system based on a Wilcox molecular torsion balance:**




$$\Delta\Delta G_{C=O\cdots C=O} = \Delta G_{(\pm)-1} - \Delta G_{(\pm)-2} - \Delta G_{(\pm)-3} + \Delta G_{(\pm)-4}$$

Fischer *et al.,* Proc. Natl. Acad. Sci. U.S.A. 105, 17293 (2008)

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# Orthogonal Multipolar Interactions

Fluorine substituents are sometimes found at short distances (3.0-3.7 Å) and orthogonal to carbonyl carbon atoms (not the most frequent arrangement for fluorine in the PDB!)

Model system in chemical double mutant cycles:

➜ weakly attractive nature of the orthogonal C-F···C=O interaction

➜ binding free enthalpy contribution in apolar environments:
$\Delta\Delta G$ = -0.2 to -0.3 kcal/mol.

Multiple SAR examples have shown:
This interaction facilitates increases in binding affinity!

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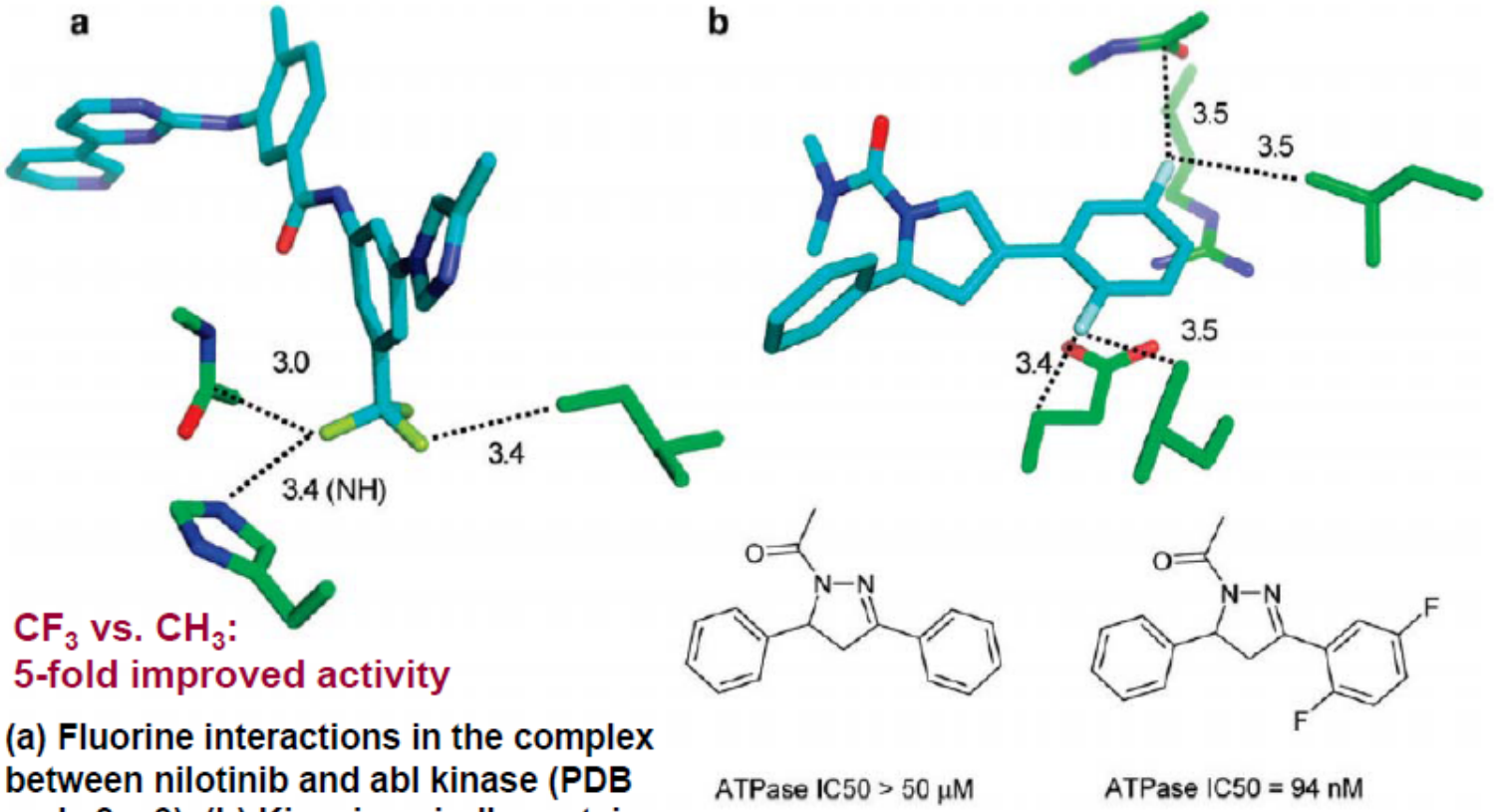
Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

# Orthogonal Multipolar Interactions




$CF_3$ vs. $CH_3$:
5-fold improved activity

ATPase IC50 > 50 µM

ATPase IC50 = 94 nM

(a) Fluorine interactions in the complex between nilotinib and abl kinase (PDB code 3cs9). (b) Kinesin spindle protein structure (2fl6) and activity data of two closely related inhibitor structures. Distances are in Å.

Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

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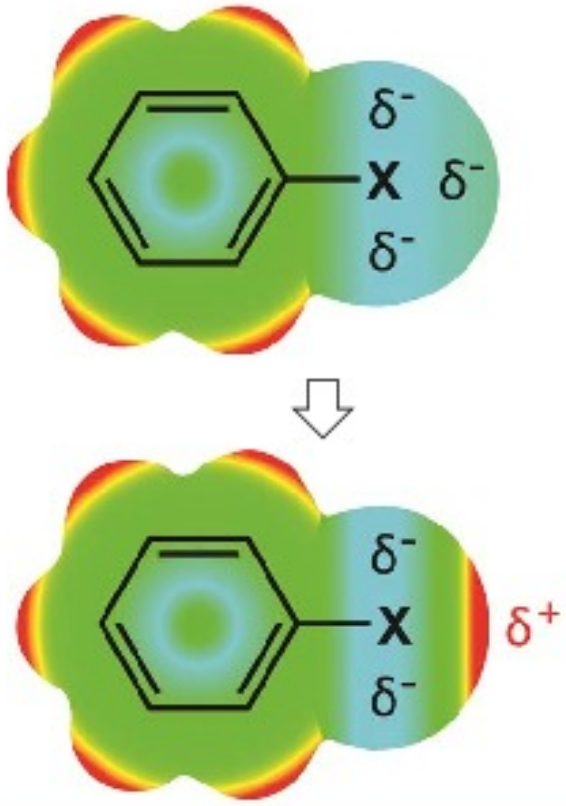
# Halogen Bonds

## HALOGEN BONDING: ANISOTROPY OF ELECTRON DISTRIBUTION





**σ - hole concept:**

**area of positive charge**
**due to deficiency of electronic charge**
**in the outer lobe of the $p_z$ orbital**
**(z is direction of C-X bond)**

T. Clark, M. Hennemann, J. Murray, P. Politzer, *J. Mol. Model.* 2007, 13, 291

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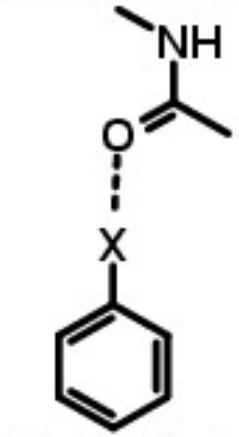
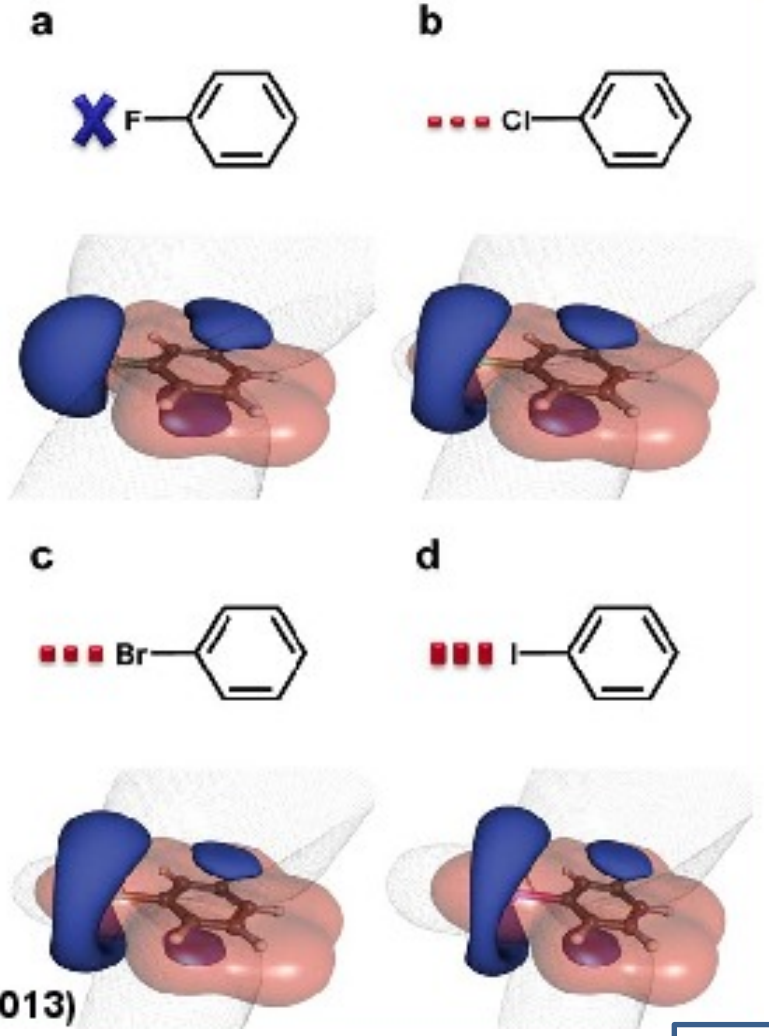
# Halogen Bonds

## ELECTROSTATICALLY-DRIVEN DIRECTIONAL INTERACTION

**Electron pair donor (such as carbonyl oxygen of backbone amide)**




**X = Cl, Br, I**




Wilcken *et al.* J. Med. Chem. 56, 1363 (2013)

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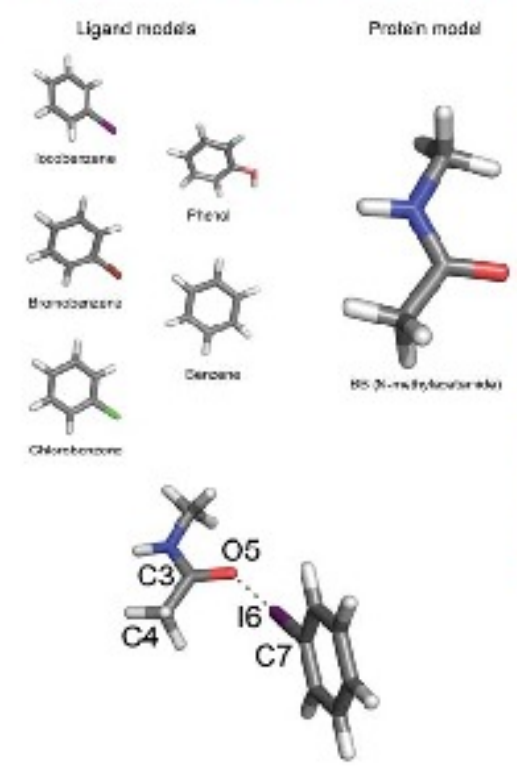
# Halogen Bonds




Wilcken *et al.*, *JCAMD* 26, 935 (2012)

| Complex | ΔE *[kJ/mol]* | Method | $d_{O5\text{-}X6/H6}$ (% vdW) | $\alpha_{C4\text{-}O5\text{-}X6/H6}$ |
|---|---|---|---|---|
| $C_6H_5I..BB$ | -14.2 | MP2/ TZVPP | 302 pm (86.3%) | 118.7° |
| $C_6H_5Br..BB$ | -9.0 | MP2/ TZVPP | 304 pm (90.2%) | 116.7° |
| $C_6H_5Cl..BB$ | -5.6 | MP2/ TZVPP | 312 pm (95.4%) | 106.7° |
| $C_6H_6..BB$ | -8.4 | MP2/ TZVPP | 230 pm (84.6%) | 107.6° |
| $C_6H_5OH..BB$ | -44.5 | MP2/ TZVPP | 174 pm (64.4%) | 114.3° |
| $C_6H_5I..BB$ | -17.6 | CCSD(T)/ CBS* | 306 pm (87.4%) | 119.4° |

* Higher order correlation energy :
(Boys and Bernardi Counterpoise Correction)

$$\Delta E_{CBS}^{CCSD(T)} = \Delta E_{CBS}^{MP2} + (\Delta E_{cc-pVTZ}^{CCSD(T)} - \Delta E_{cc-pVTZ}^{MP2})$$

Complete basis set extrapolation (Halkier):

$$\Delta E_{CBS}^{MP2} = \frac{\Delta E_X^{MP2} X^3 - \Delta E_Y^{MP2} Y^3}{X^3 - Y^3}$$

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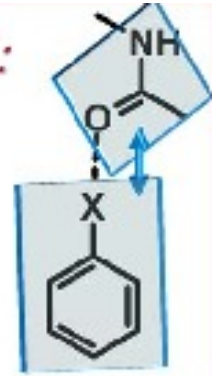
# Halogen Bonds

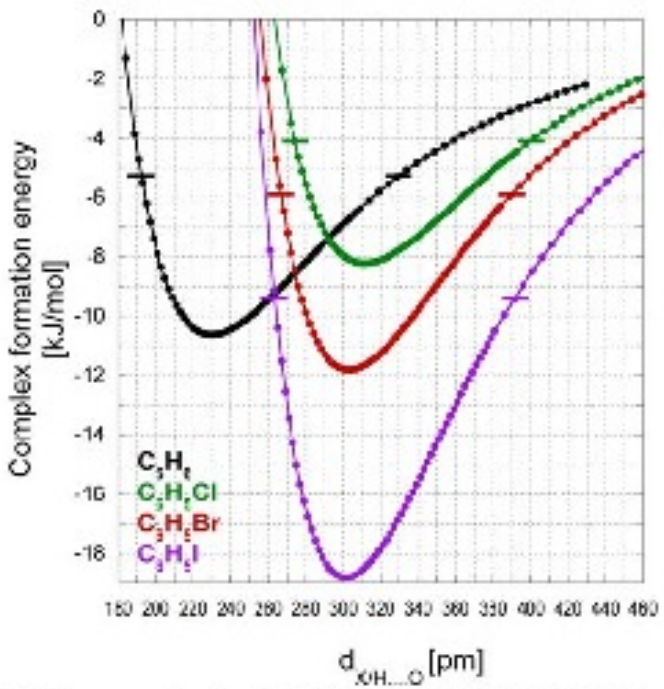
## VARIATION OF INTERACTION GEOMETRIES:

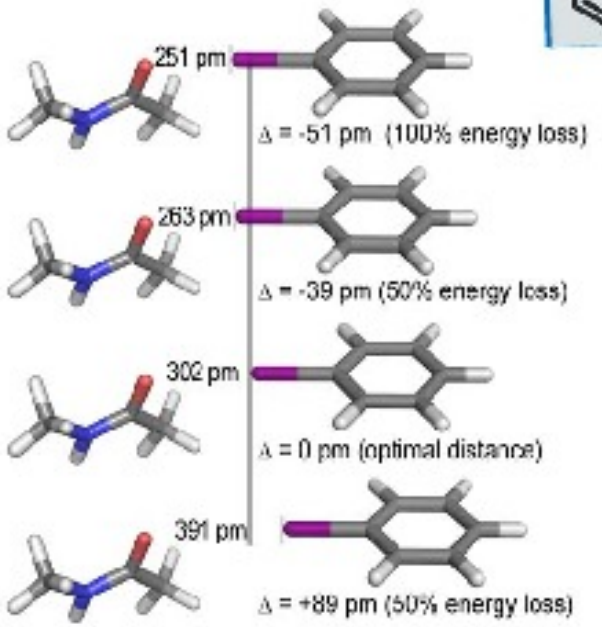



**MP2 / def2-TZVPP geometries:**

➜ optimal distances are significantly below sum of van der Waals radii

➜ interaction changes significantly on a picometer scale!



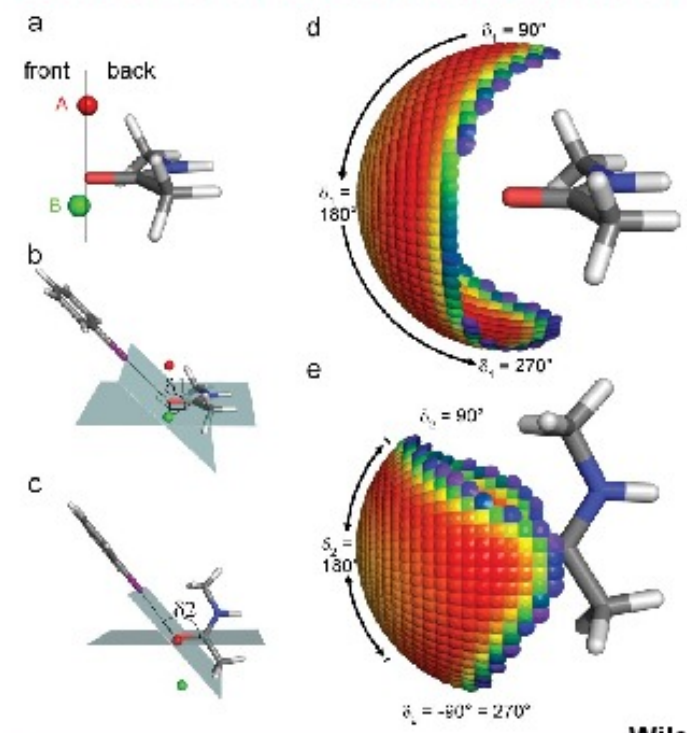



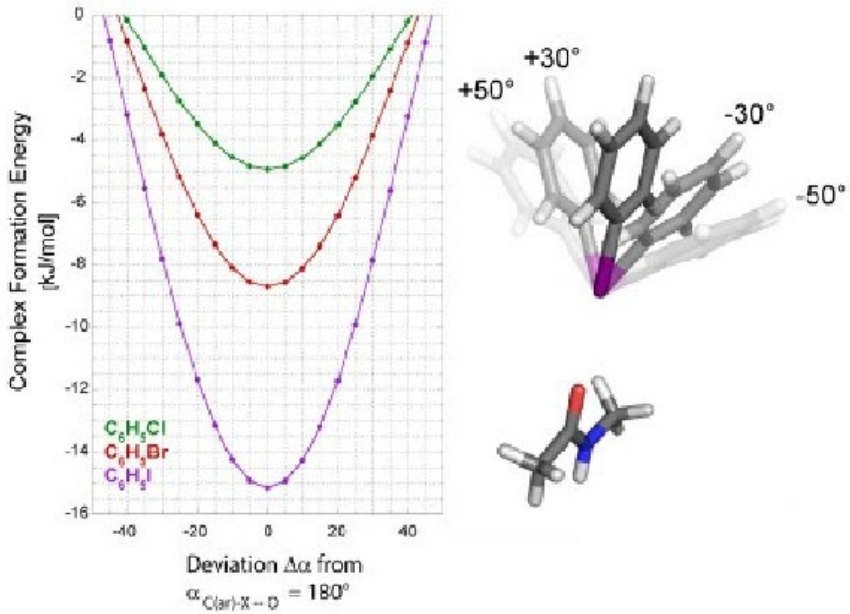

Wilcken *et al.*, *JCAMD* 26, 935 (2012)

➜ „Pico-Pharmacology“

**22**

# Halogen Bonds
# Variation of Interaction Geometries




*ANGULAR DEPENDENCY*:

➜ semi-directional

➜ tolerance: out of plane > in plane

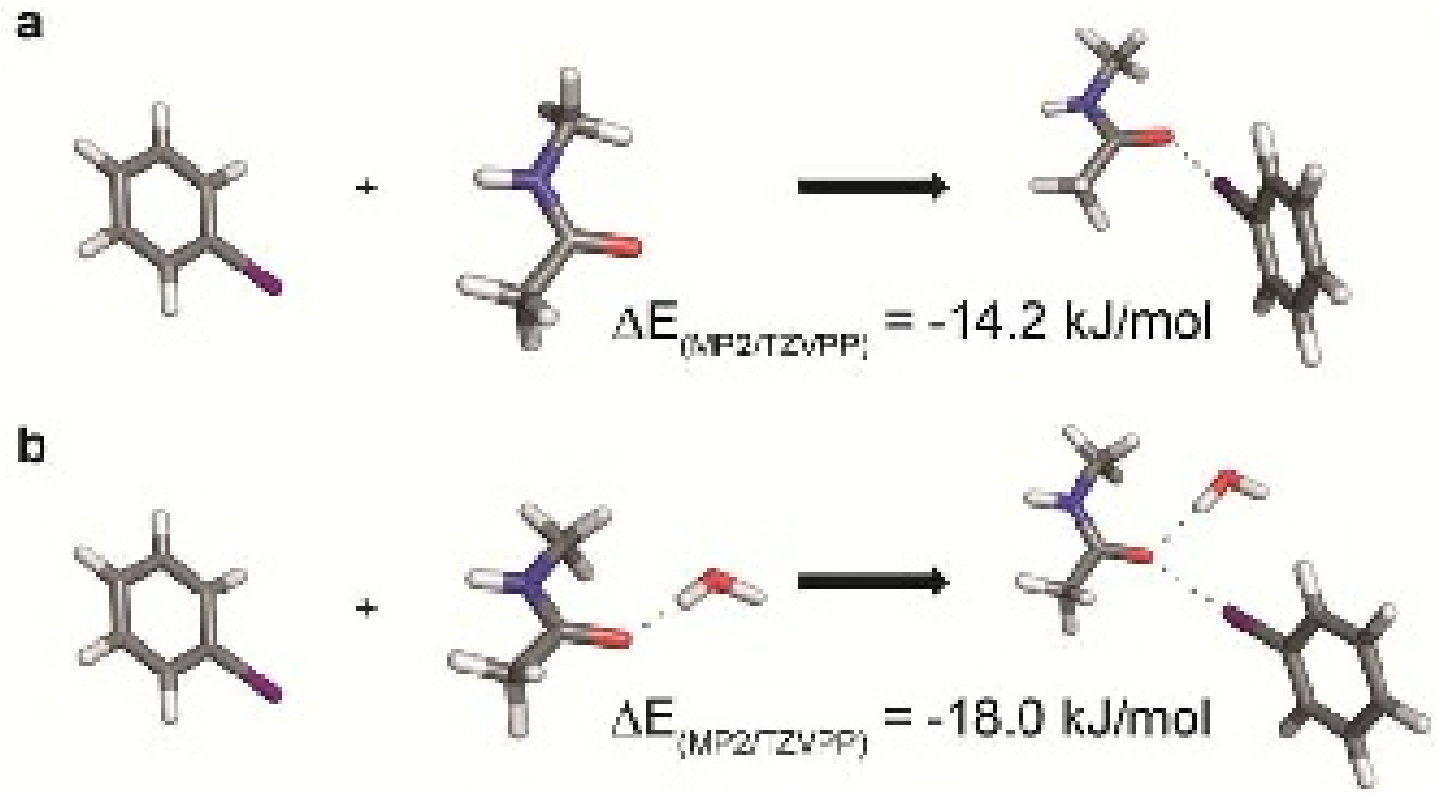
*σ-HOLE DEPENDENCY*:

➜ only small deviations from 180° are tolerated

Wilcken *et al.*, *JCAMD* 26, 935 (2012)

**23**

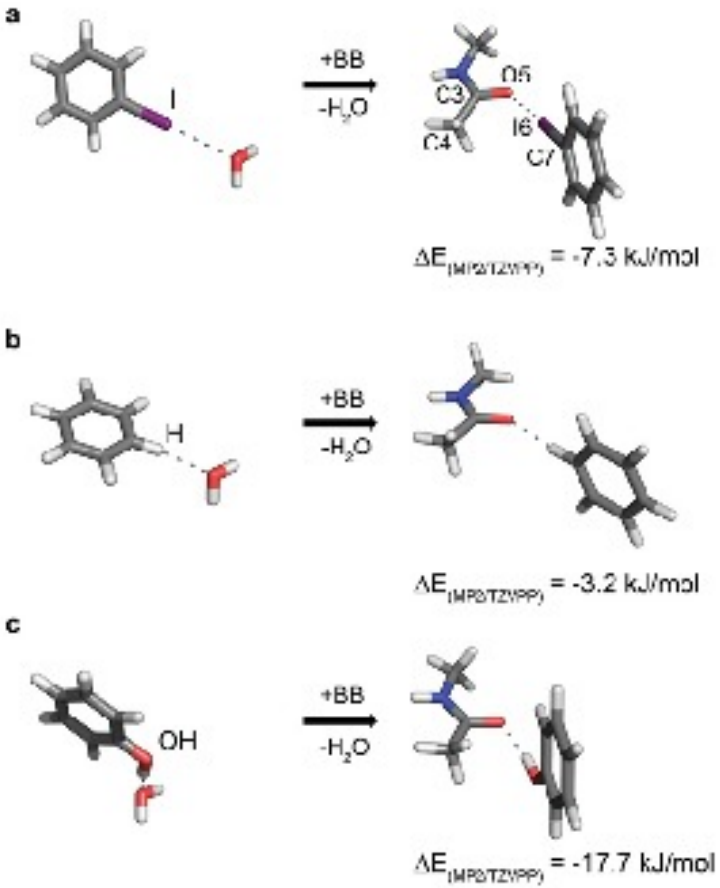
# Cooperativity of Halogen Bonds and Hydrogen Bonds




Strength of halogen bonds increase when water is bound simultaneously!

24

# Simple Model of Desolvation Effects:

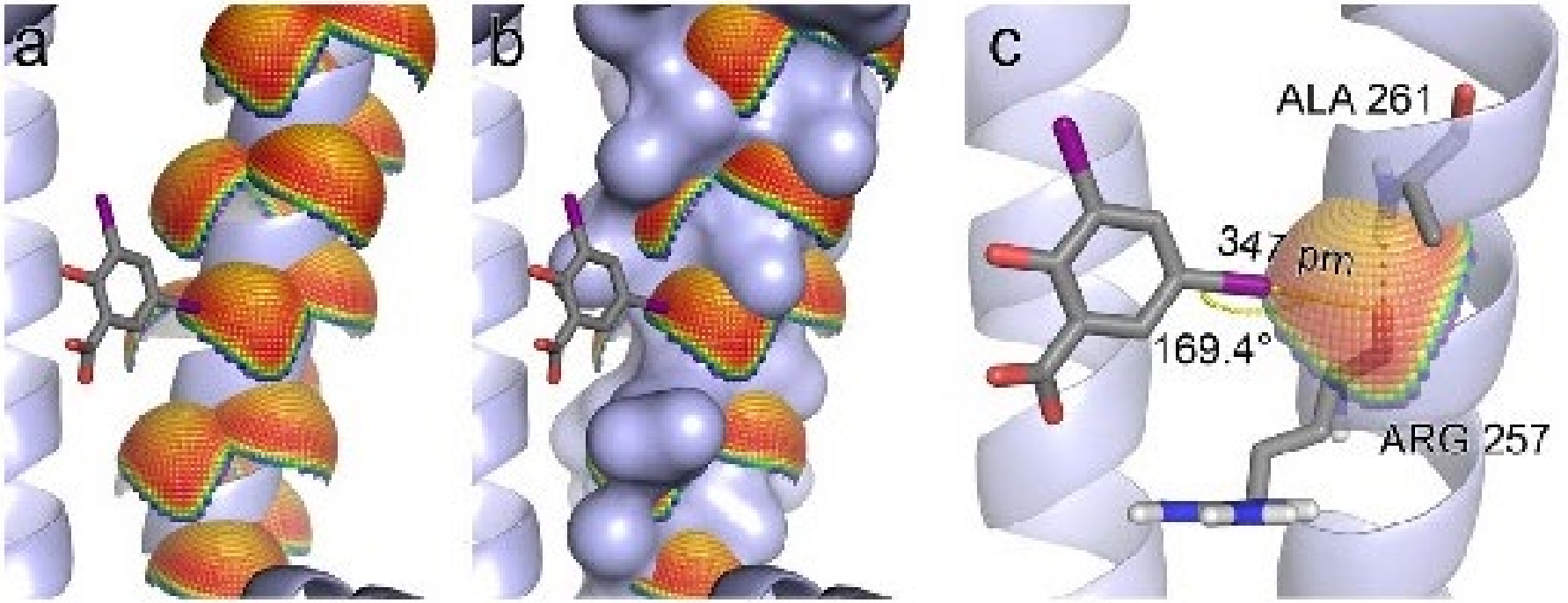



One water molecule is substituted by the backbone model system

Halogen bonding to BB carbonyl is superior to weak hydrogen bonding, but not to moderately strong hydrogen bonding when taking ligand desolvation into account!

**24**

# Halogen Bonds Interaction Partners



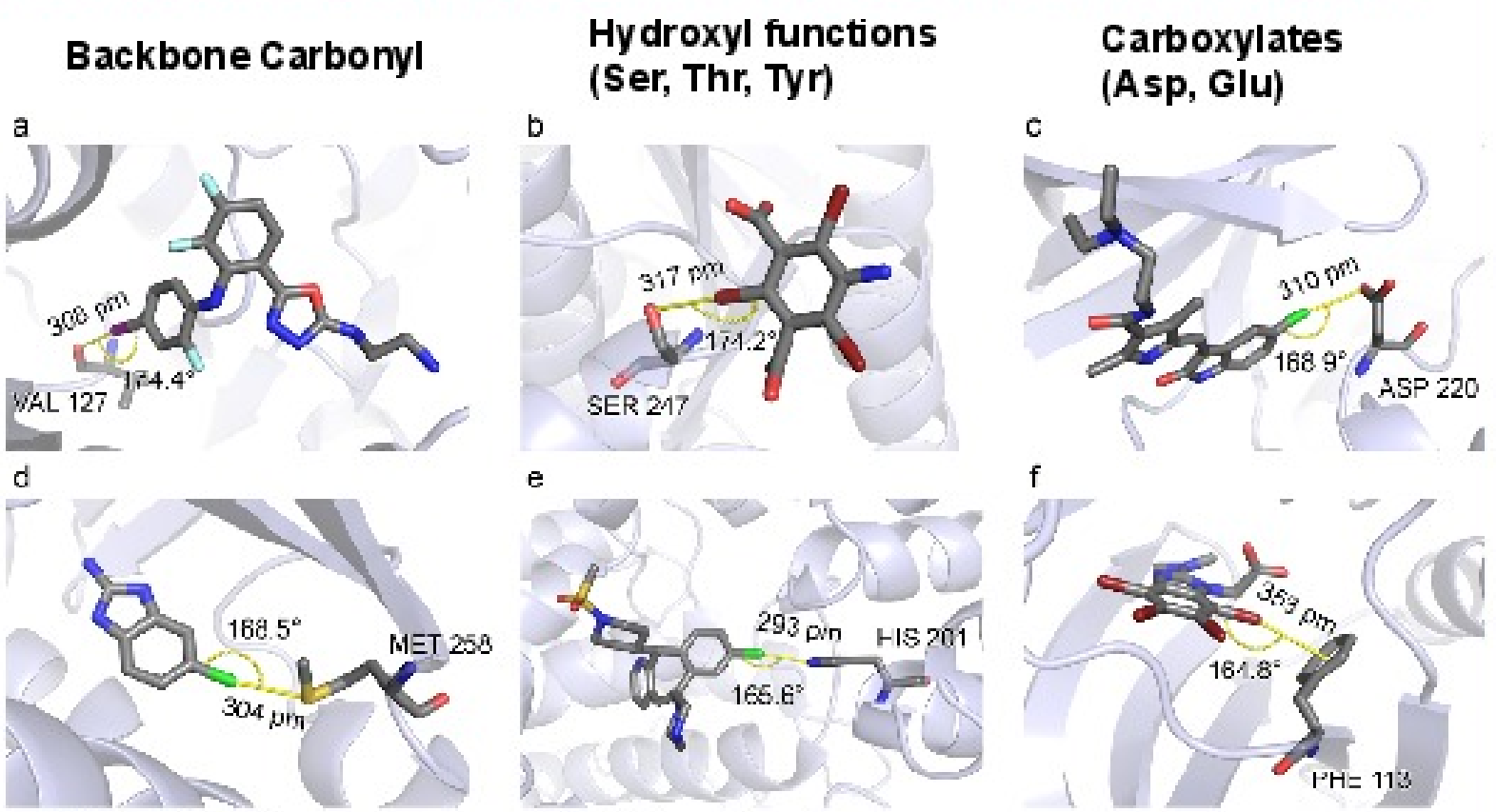

α-helix:

- backbone carbonyl is well accessible
- size and orientation of the side chains are crucial

Wilcken *et al.* J. Med. Chem. 56, 1363 (2013)

25

# Halogen Bonds Interaction Partners




Wilcken *et al.* J. Med. Chem. 56, 1363 (2013)

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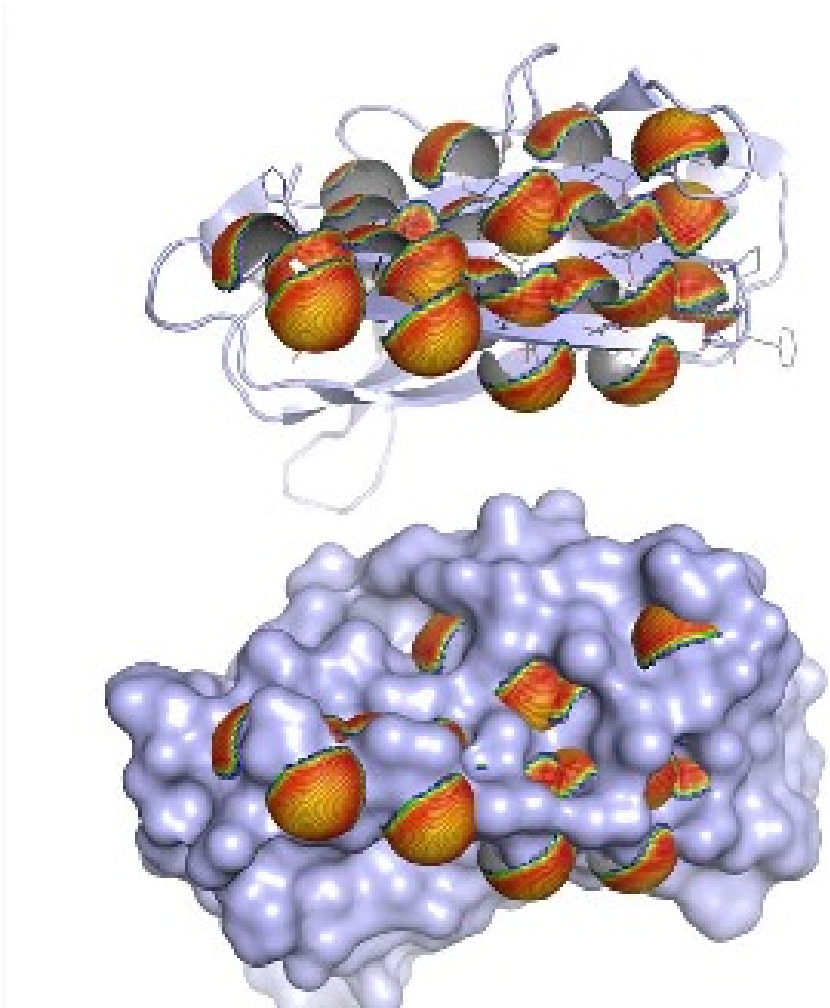
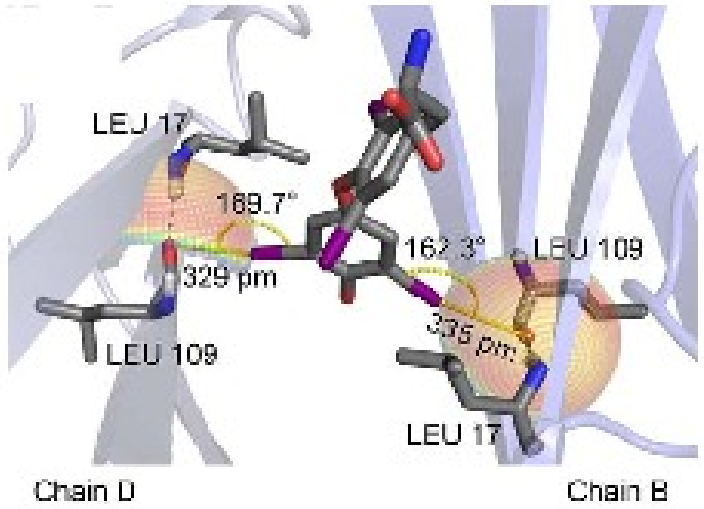
# Protein Structure Accessibility for Halogen Bonds






β-sheet:

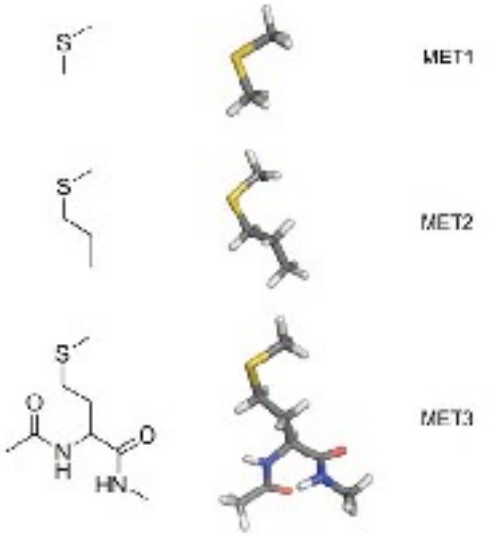
- backbone carbonyl is accessible (benefits from tilted β-sheet!)
- size and orientation of the side chains are crucial

Wilcken *et al.* J. Med. Chem. 56, 1363 (2013)

**27**

# Halogen Bonds

## HALOGEN-SULFUR CONTACTS:




| Complex | ΔE [kJ/mol] | Method | $d_{S5\text{-}X6/H6}$ (% vdW) | $\alpha_{S5\text{-}X6/H6\text{-}C7/O7}$ |
|---|---|---|---|---|
| $C_6H_5I$..MET1 | -19.3 | MP2/ QZVPP | 336 pm (88.9%) | 169.8° |
| $C_6H_5Br$..MET1 | -13.0 | MP2/ QZVPP | 343 pm (94.0%) | 170.1° |
| $C_6H_5Cl$..MET1 | -10.1 | MP2/ QZVPP | 348 pm (98.0%) | 162.7° |
| $C_6H_6$..MET1 | -11.0 | MP2/ QZVPP | 279 pm (96.2%) | 156.9° |
| $C_6H_5OH$..MET1 | -29.1 | MP2/ QZVPP | 226 pm (77.9%) | 153.8° |
| $C_6H_5I$..MET1 | -17.3 | CCSD(T)/ CBS* | 345 pm (91.3%) | 168.4° |

* Higher order correlation energy :
(Boys and Bernardi Counterpoise Correction)

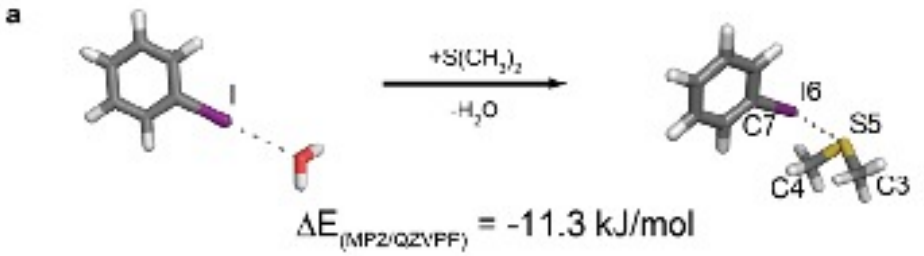
$$\Delta E_{CBS}^{CCSD(T)} = \Delta E_{CBS}^{MP2} + (\Delta E_{cc-pVTZ}^{CCSD(T)} - \Delta E_{cc-pVTZ}^{MP2})$$

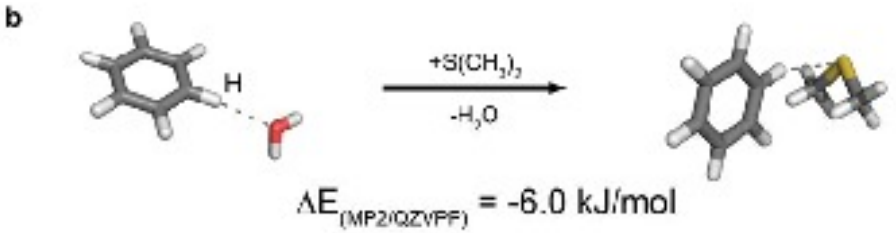
**Wilcken R., Zimmermann M. O., Lange A., Zahn S., Kirchner B., Boeckler F. M., *JCTC* 2011, 7, 2307–2315**

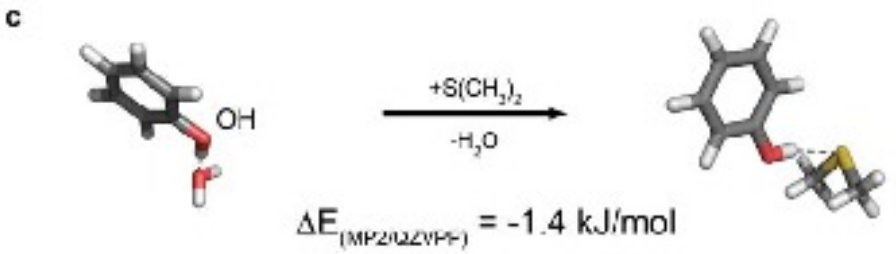

**28**

# Halogen Bonds

## DESOLVATION EFFECTS IN MET-HALOGEN BONDING:




**Halogen bonding to MET sulfur is superior to weak and moderately strong hydrogen bonding when taking ligand desolvation into account!**

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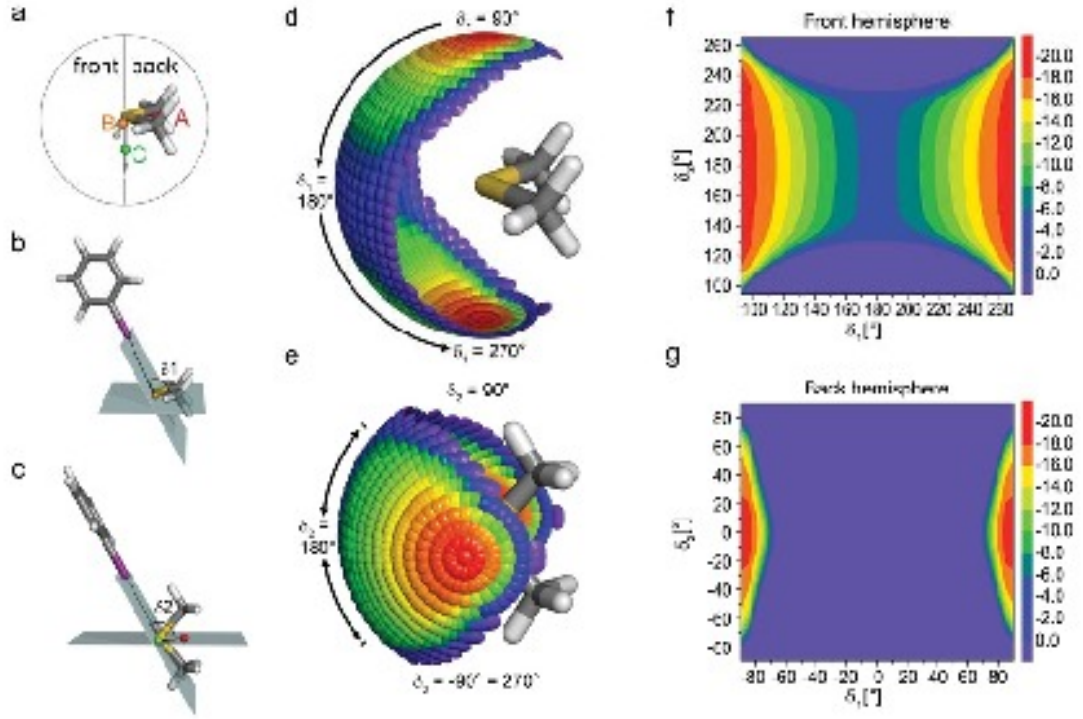
# Halogen Bonds

## SPHERICAL DEPENDENCY OF MET-HALOGEN BONDING :

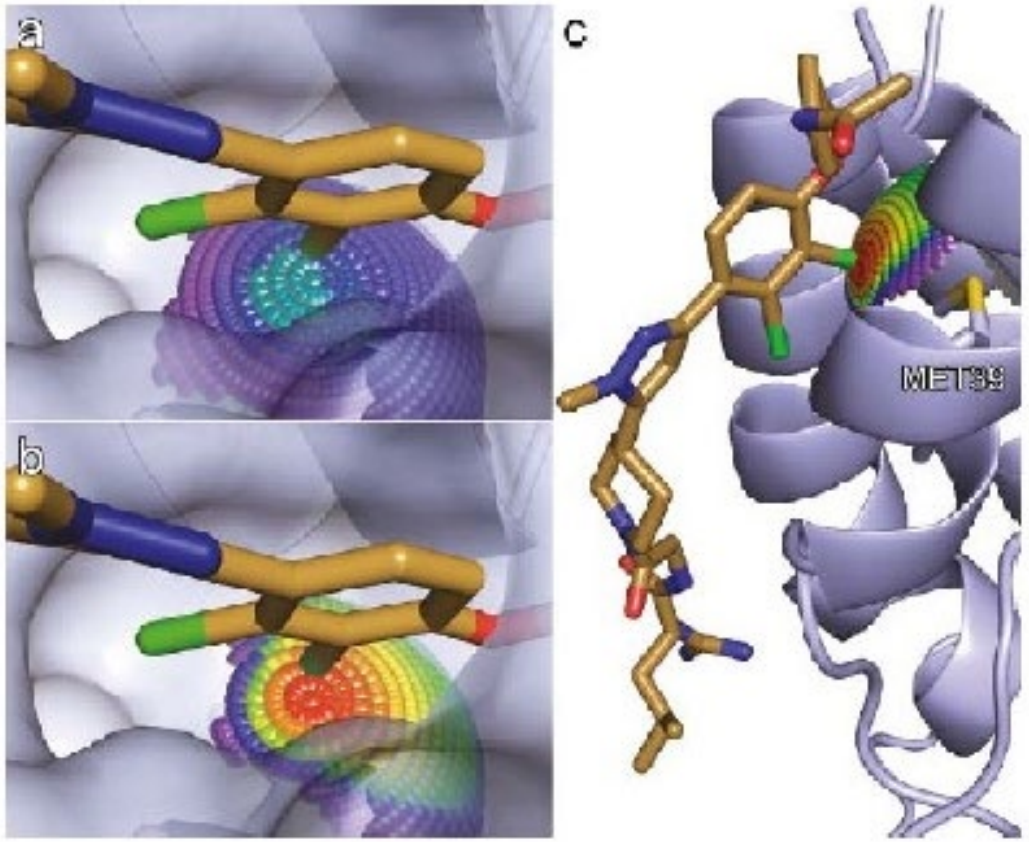



Halogen Bonding toward MET is energetically favorable only in distinct interaction areas → MET must be hit precisely in the right geometry

30

# Halogen Bonds

## SPHERICAL DEPENDENCY OF MET-HALOGEN BONDING :




**IL-2 with the bound ligand SP4160**

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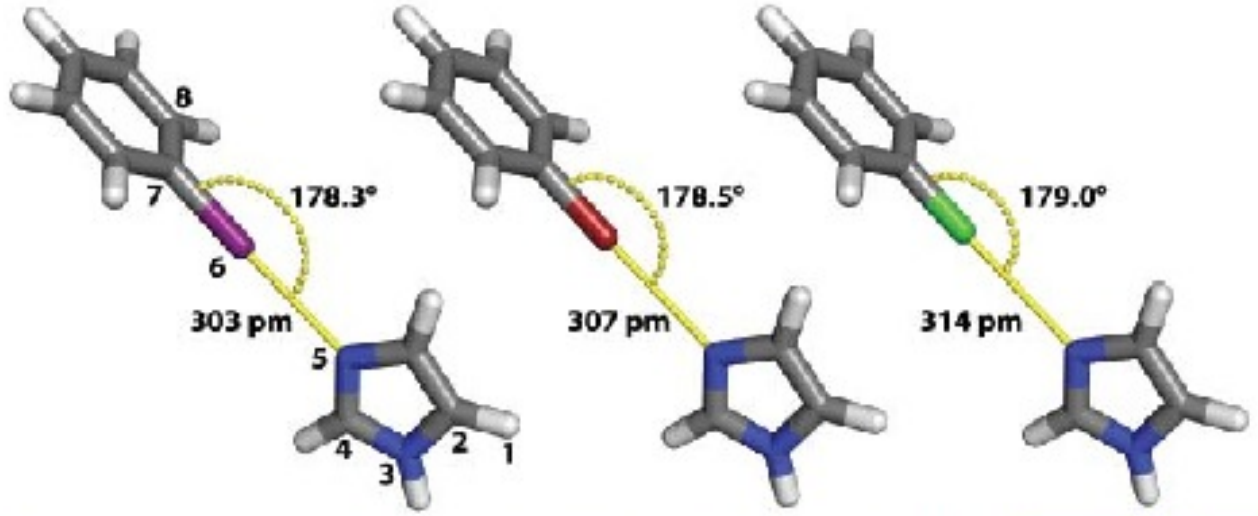
# Halogen Bonds

## HALOGEN BONDING TO HISTIDINE:




| Method | BP86(RI)-D | BLYP(RI)-D | TPSS(RI)-D | B3LYP-D | MP2 |
|---|---|---|---|---|---|
| Basis Set | def2-TZVPP | def2-TZVPP | def2-TZVPP | def2-TZVPP | def2-TZVPP |
| **ΔE** Adduct formation [kJ/mol] | -18.9 | -17.7 | -19.7 | -17.5 | -20.4 / -16.0* |
| d(N5 - I6) [pm] | 298 | 311 | 299 | 311 | 303 |
| α1(C4-N5-I6) [°] | 131.0 | 131.5 | 113.6 | 131.8 | 132.8 |
| α2(N5-I6-C7) [°] | 177.7 | 177.6 | 175.2 | 177.7 | 178.3 |
| δ1(N3-C4-N5-I6) [°] | -178.4 | -178.0 | 1.9.9 | -178.0 | -177.6 |

Results for optimization of the Iodobenzene Imidazole adduct system using different methods.
*BSSE corrected energy

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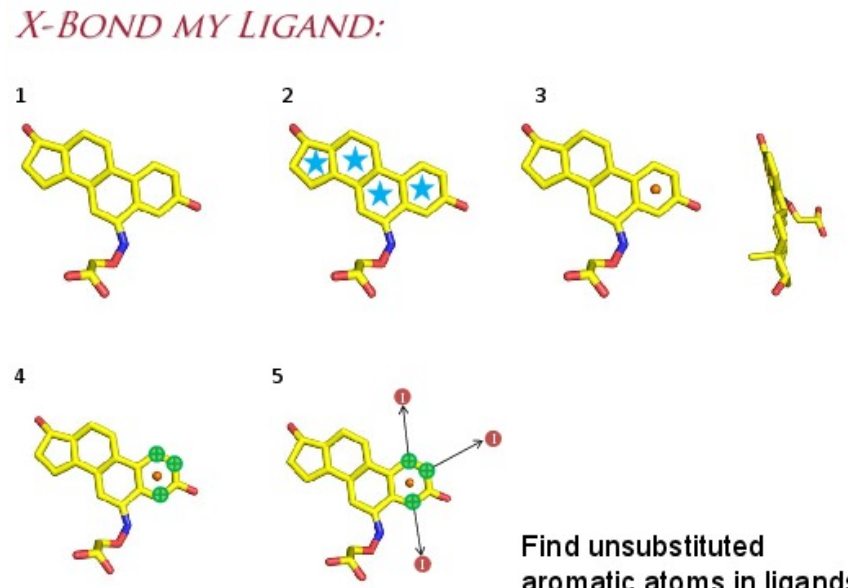
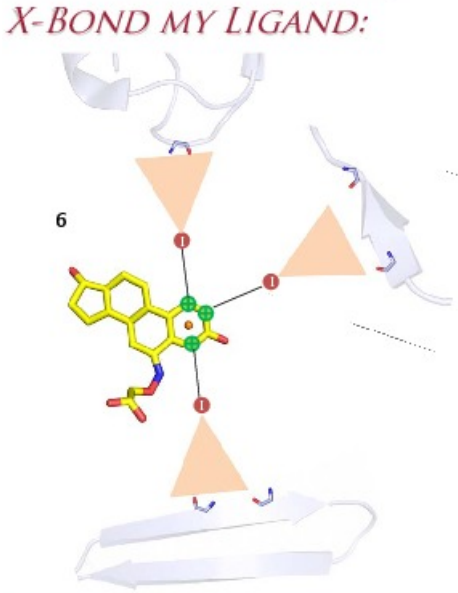
# Halogen Bonds – X-Bond My Ligand




Find unsubstituted aromatic atoms in ligands




Scoring of putative halogen placement

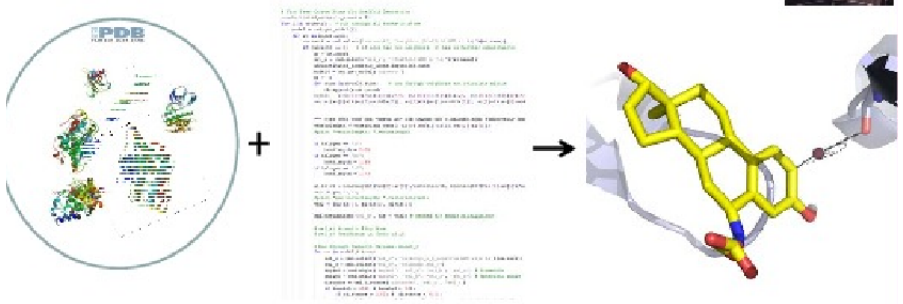
Variables:

Distance to carbonyl oxygen

σ-hole

Spatial orientation of halogen around carbonyl

Scaffold decoration with halogen atoms




Protein Data Bank ~79000 structures

Python/PyMol-Script

Possible Halogen Bonds

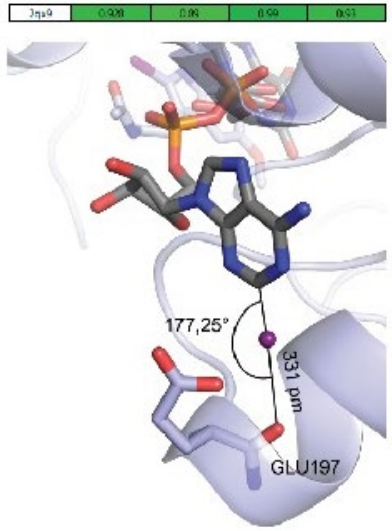

**HBScore** combines *distance*, *spherical orientation* and *σ-hole* in one score

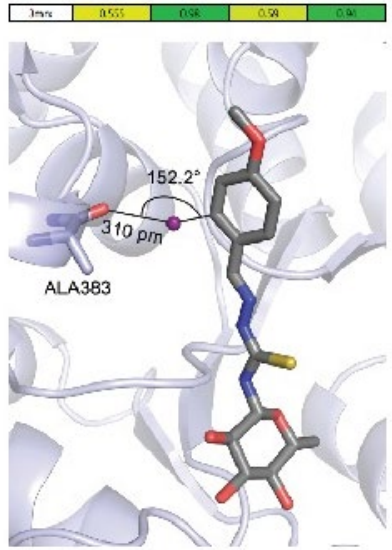
Halogens: Iodine, Bromine and Chlorine

ClashScore for Protein and Ligand

Check for sterical clashes with the protein → Evaluate geometry and calculate score

# Halogen Bonds – X-Bond My Ligand




Small excerpt from the output

| PDB | HBScore | Score distance | Score σ-hole | Score spherical | Clash protein | Clash ligand | Distance | σ-hole | Type |
|---|---|---|---|---|---|---|---|---|---|
| 1x9q | 0.945 | 1 | 0.99 | 0.96 | 6.4 | 0 | 3.022 | 175.711 | IMMUNE SYSTEM |
| 2qx9 | 0.938 | 0.99 | 0.99 | 0.93 | 29.8 | 0 | 3.308 | 177.249 | OXIDOREDUCTASE |
| 2v13 | 0.752 | 0.95 | 0.84 | 0.9 | 4.8 | 4.2 | 3.197 | 163.015 | HYDROLASE |
| 1ydk | 0.749 | 0.96 | 0.83 | 0.9 | 7.5 | 36.3 | 3.468 | 163.772 | TRANSFERASE |
| 3f15 | 0.737 | 0.09 | 0.99 | 0.74 | 64.3 | 0 | 5.368 | 178.665 | ISOMERASE |
| 1lwe | 0.636 | 0.85 | 0.87 | 0.72 | 15.6 | 4.3 | 3.670 | 164.709 | TRANSFERASE |
| 2x8p | 0.579 | 0.8 | 0.7 | 0.83 | 11.8 | 5.5 | 3.440 | 156.917 | TRANSFERASE |
| 3rmm | 0.555 | 0.98 | 0.59 | 0.94 | 0 | 0 | 3.088 | 152.201 | TRANSFERASE |
| 1ha2 | 0.474 | 0.07 | 0.97 | 0.49 | 48.2 | 0 | 5.602 | 172.919 | TRANSPORT PROTEIN |
| 2rd0 | 0.451 | 0.35 | 0.59 | 0.77 | 25.6 | 0 | 4.228 | 152.154 | HYDROLASE |
| 2wmd | 0.321 | 0.3 | 0.54 | 0.6 | 6.6 | 0 | 4.371 | 150.162 | OXIDOREDUCTASE |
| 3IIC | 0.288 | 0.18 | 0.55 | 0.52 | 46.1 | 3.4 | 4.811 | 150.729 | MEMBRANE PROTEIN |
| 1i41 | 0.149 | 0.08 | 0.35 | 0.43 | 1.6 | 0 | 2.433 | 143.469 | LYASE |
| 2ozr | 0 | 0.11 | 0.32 | 0 | 57.8 | 0 | 5.236 | 142.749 | HYDROLASE |
| 3cuk | 0 | 0.05 | 0.51 | 0 | 60.1 | 0 | 5.925 | 140.305 | OXIDOREDUCTASE |

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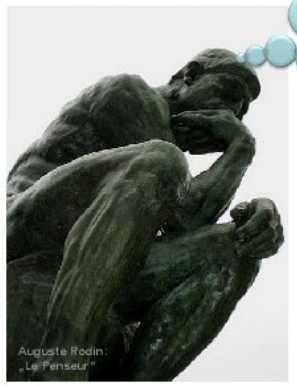
# Halogen Bonds

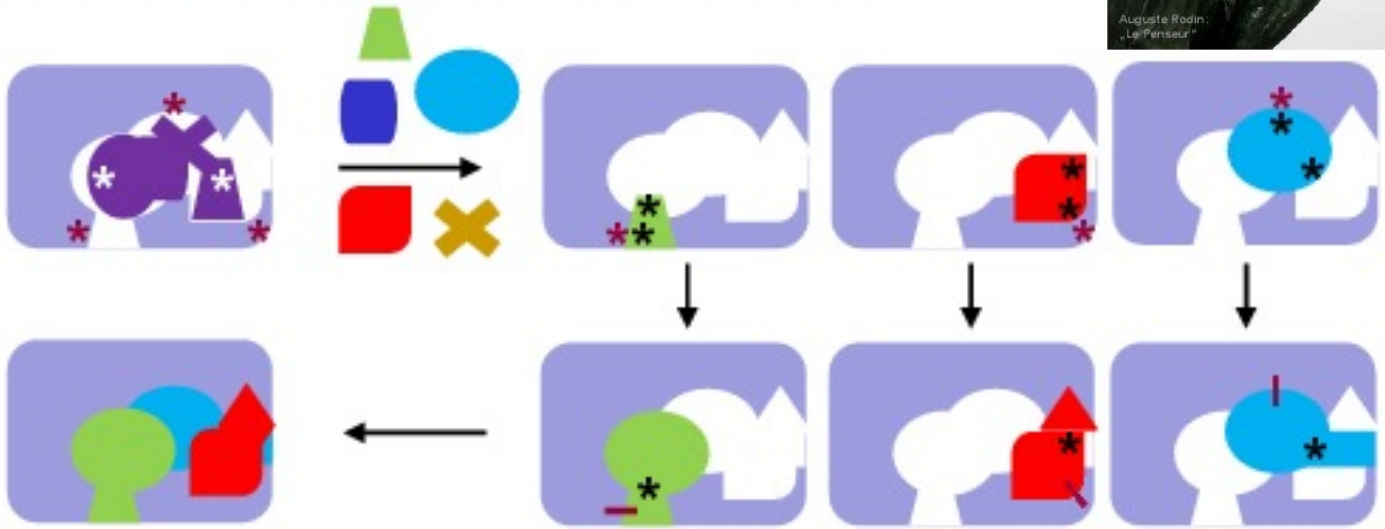



How can we make use of this knowledge ?

**HEFLibs**

Exploiting halogen bonding for lead placement by halogen-enriched fragment libraries

## FRAGMENT-BASED SCREENING:



**Use of halogen-enriched fragments:**

- **Only few interactions per fragment possible**
  **➜ increased chance to find binding modes based on halogen bonds!**
- **Additional halogen-atoms facilitate easy extention by cross-coupling reactions**

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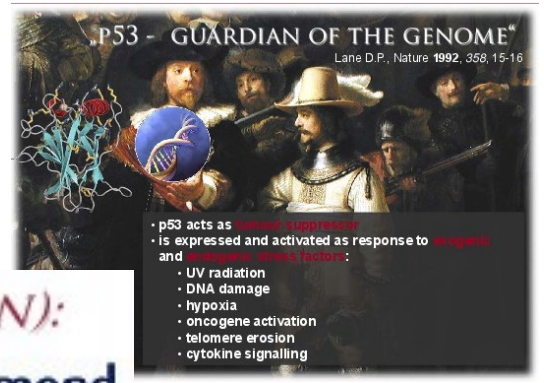
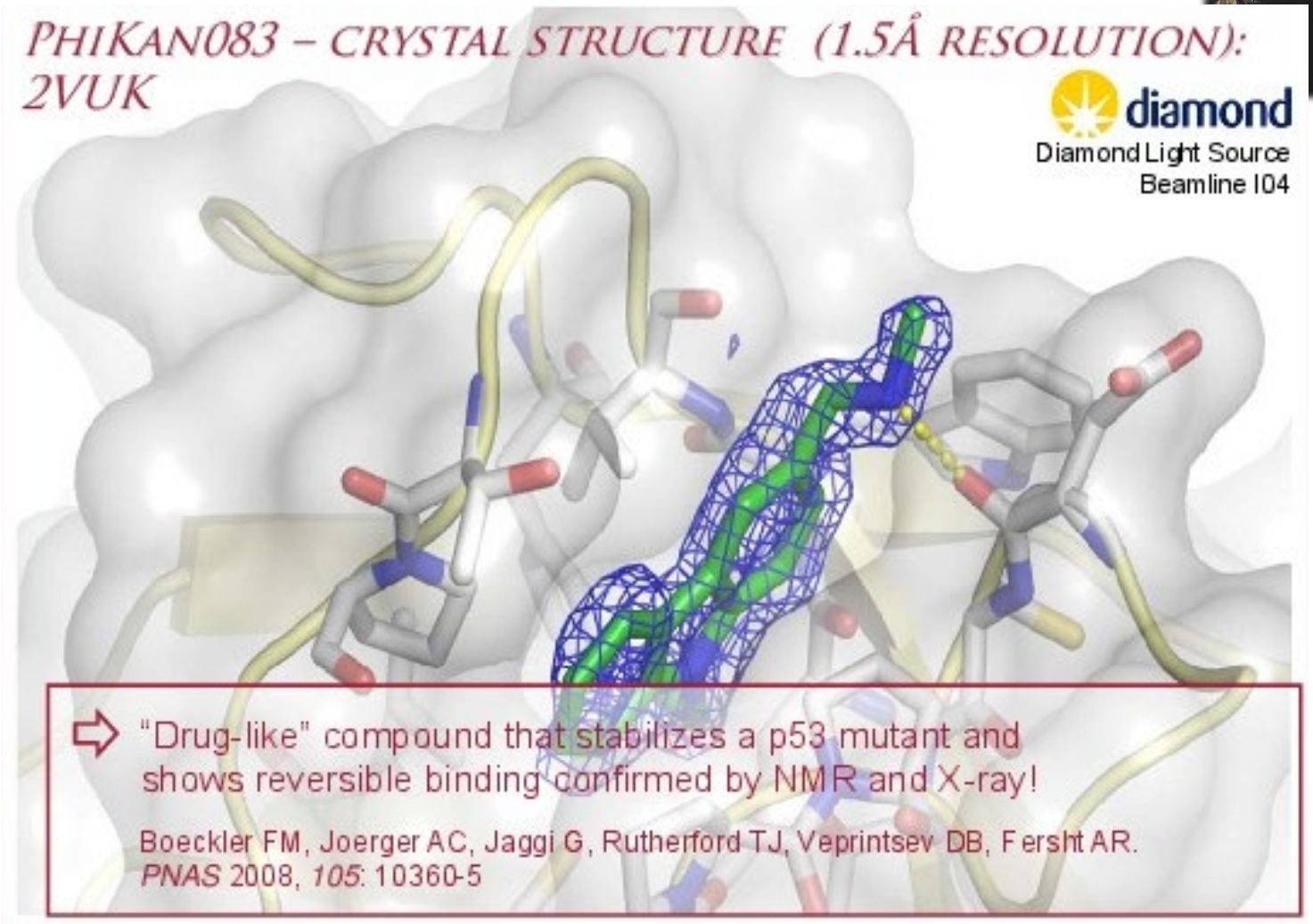
# Halogen Bonds

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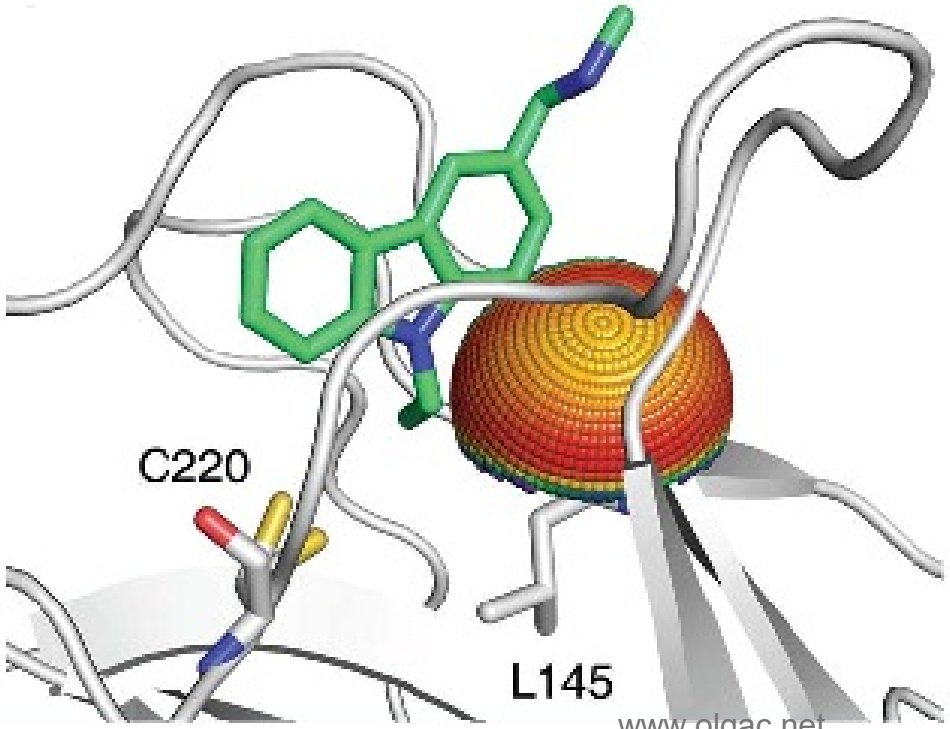
## PHIKAN083 – CRYSTAL STRUCTURE (1.5Å RESOLUTION): 2VUK

diamond
Diamond Light Source
Beamline I04

**Network of interacting waters gives hints about possible improvements:**

**➜ Addressing Leu145 (end of the β-sheet!) by HB or XB?**

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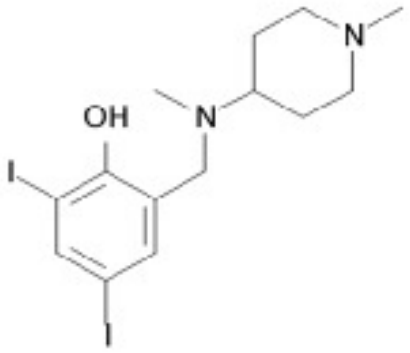
# Halogen Bonds

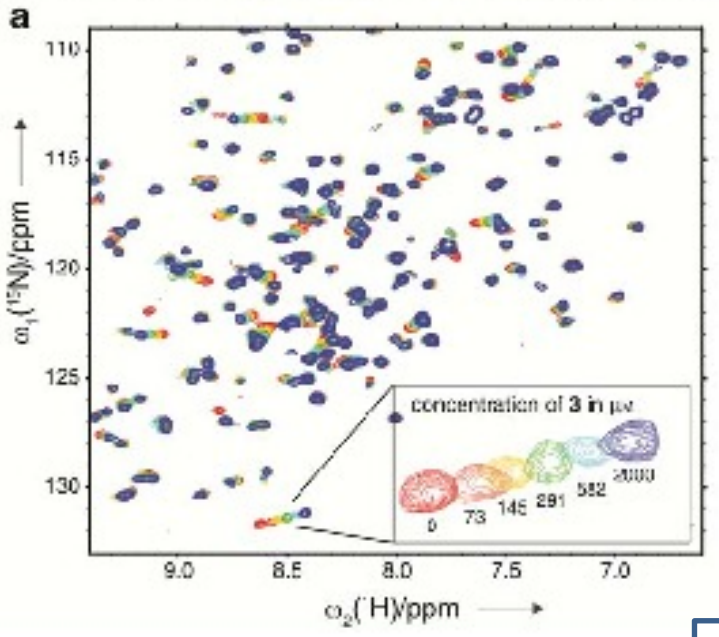
## First Design of Target-Focused HEFLibs:

I) Size: Only molecules with less than 25 heavy atoms
II) Ring count At least one aromatic ring, maximum of three rings
III) Halogens: At least one chlorine, bromine or iodine
IV) Solubility: Must contain protonatable amine or carboxylate function
V) Phenols or Anilines: Deduced from previous hits

**Screening of HEFLibs (79 compounds) yielded 10 hits. Most promising: PK784**




PK784

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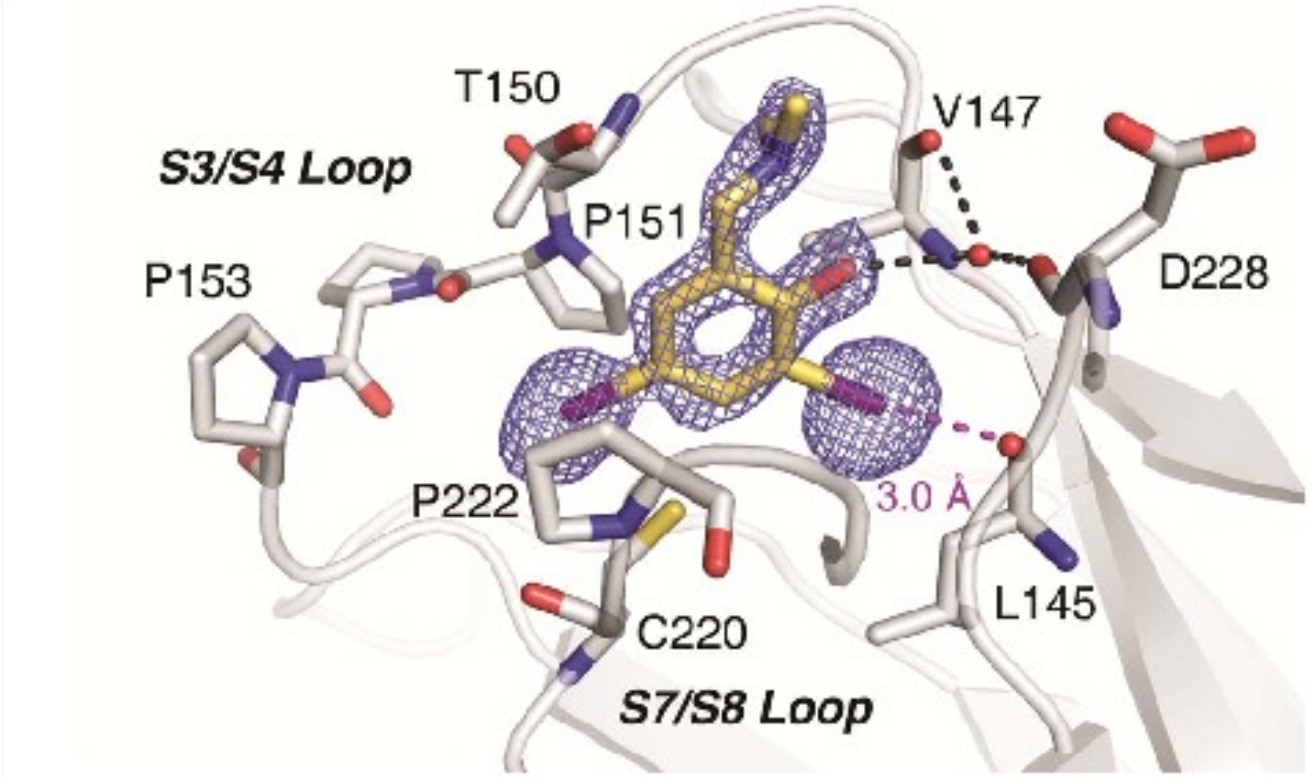
# Halogen Bonds

STRUCTURAL ANALYSIS OF PHIKAN 784 (PDB-ID: 4AGL) :




Almost optimal X-bond geometry + stabilization by interstitial water

Wilcken R., ..., Boeckler F. M., *J. Am. Chem. Soc.* 2012, 134, 6810−6818

JACS-Spotlight: *J. Am. Chem. Soc.* 2012, 134, 7195

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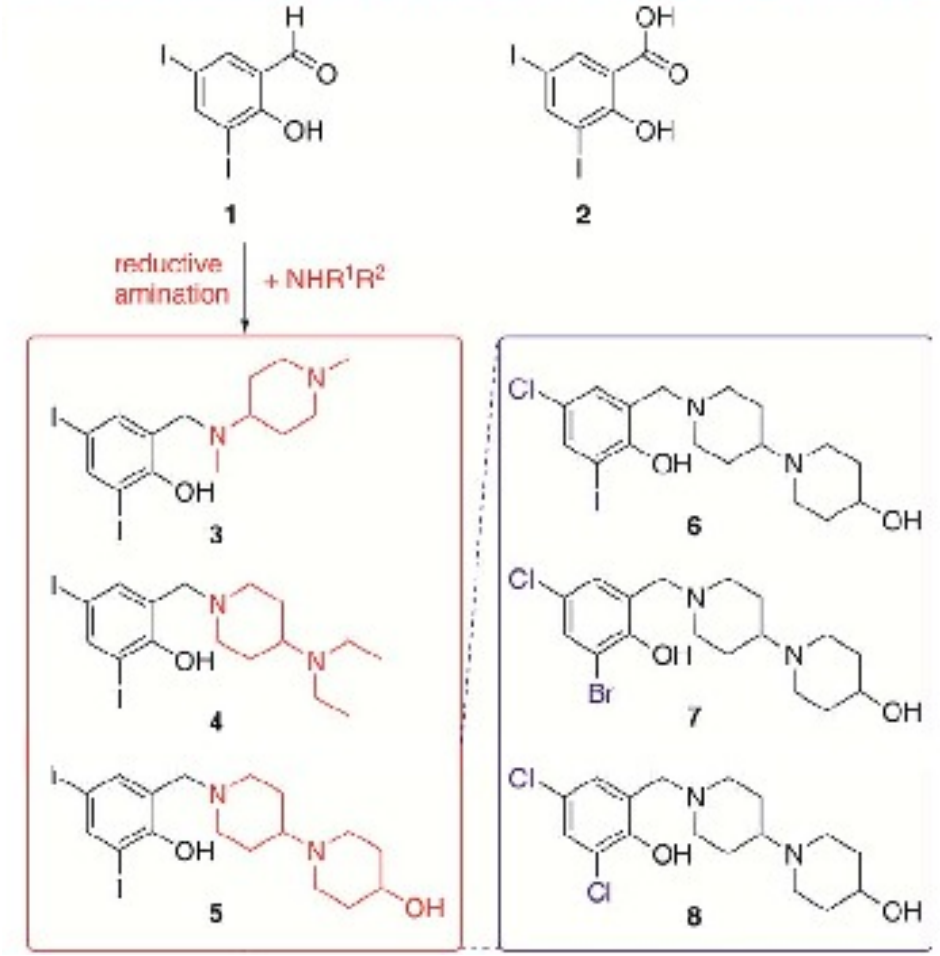
# Halogen Bonds

## SYNTHESIS STRATEGY FOR SAR STUDIES OF PK784:




Variation of amines:

Co-optimization of :

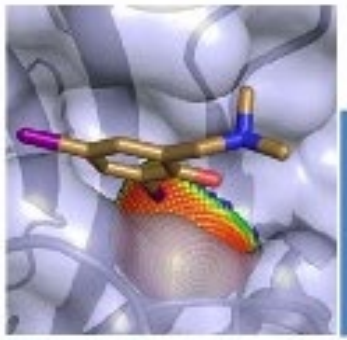
- Affinity
- Mutant Stabilization
- Solubility
- Feasibility to crystallize protein-ligand complexes

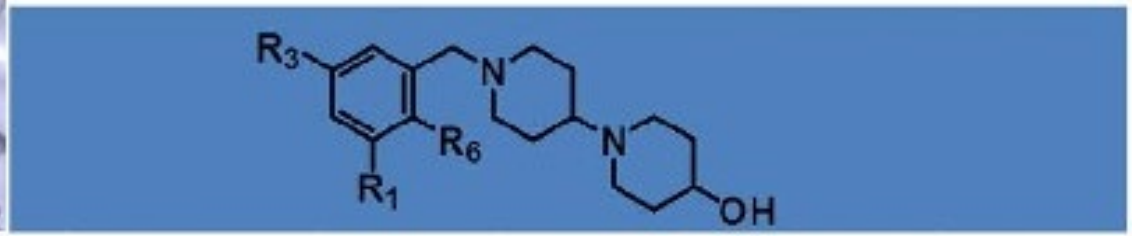
Variation of halogens:

Experimental evaluation of halogen bond contributions to target affinity

# Halogen Bonds

## Evaluation of Halogen-bond Strength:



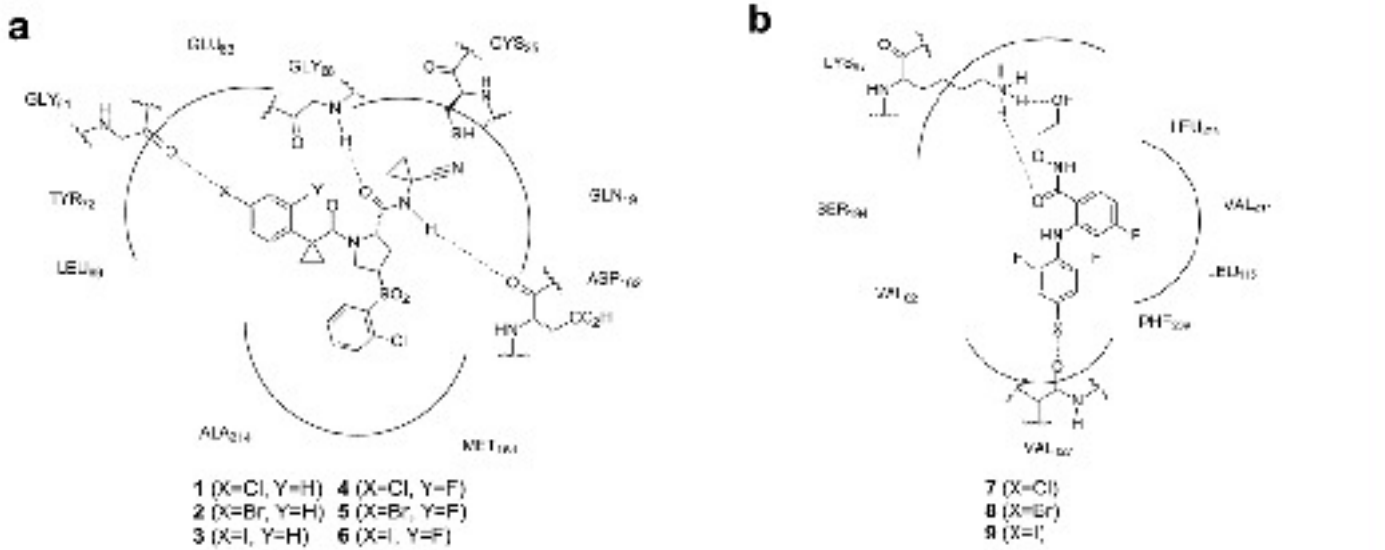



| PK# | $R_1$ | $R_3$ | $R_6$ | $K_D$ NMR [μM] | $\Delta G^{Exp}$ [kJ/mol] | $\Delta\Delta G^{Exp}$ to $R_1$=Cl [kJ/mol] | $\Delta E$ (MP2/TZVPP) [kJ/mol] | $\Delta\Delta E$ to $R_1$=Cl [kJ/mol] |
|---|---|---|---|---|---|---|---|---|
| 5151 | Cl | Cl | OH | 4900±2300 | -13.0 | --- | -5.6 | --- |
| 5149 | Br | Cl | OH | 1035±162 | -16.8 | -3.8 | -9.0 | -3.4 |
| 5121 | I | Cl | OH | 247±44 | -20.2 | -7.3 | -14.2 | -8.6 |

# Halogen Bonds

## EVALUATION OF HALOGEN-BOND STRENGTH:

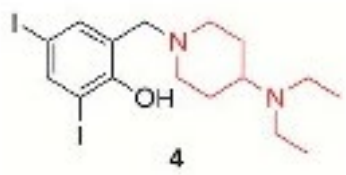
| | | hCatL inhibitors | | | | MEK1 inhibitors | |
|---|---|---|---|---|---|---|---|
| halogen | calculations, MP2/TZVPP $\Delta\Delta E^b$ (kJ/mol) | $IC_{50}$ [c] ($\mu$M) | $\Delta\Delta G^d$ (kJ/mol) | $IC_{50}$ [e] ($\mu$M) | $\Delta\Delta G^d$ (kJ/mol) | $IC_{50}$ [f] ($\mu$M) | $\Delta\Delta G^d$ (kJ/mol) |
| Cl | 0.0 | 0.022 | 0.0 | 0.03 | 0.0 | 0.026 | 0.0 |
| Br | −3.4 | 0.012 | −1.5 | 0.0065 | −3.8 | 0.0077 | −3.0 |
| I | −8.6 | 0.0065 | −3.0 | 0.0043 | −4.8 | 0.002 | −6.4 |



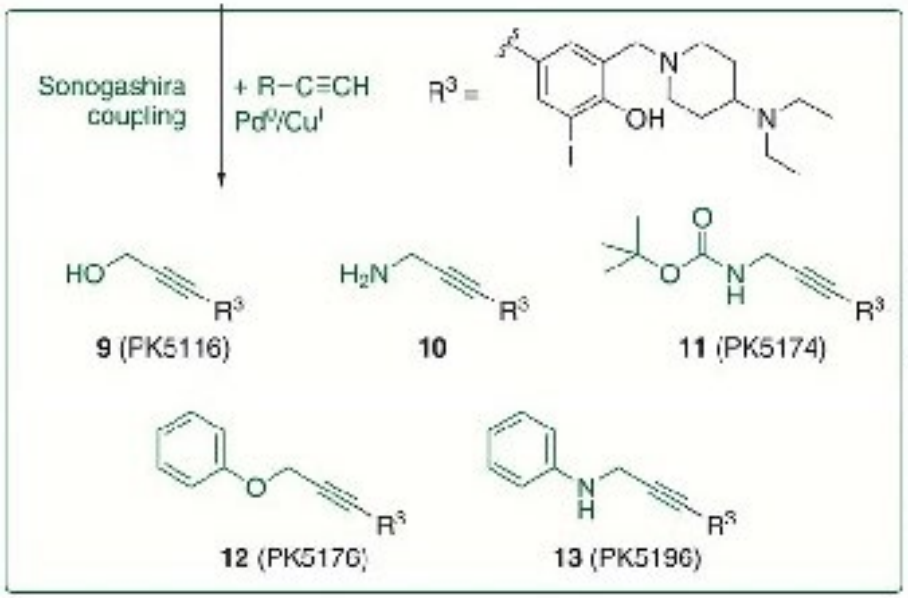

Hardegger et al., Angew. Chem., Int. Ed. 50, 314 (2011)
Hardegger et al., ChemMedChem, 6, 2048 (2011)

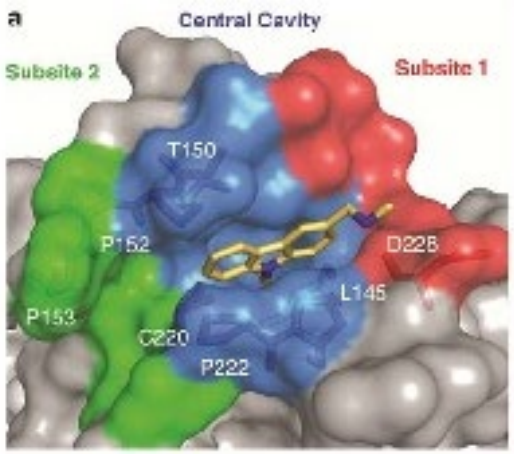
# Halogen Bonds

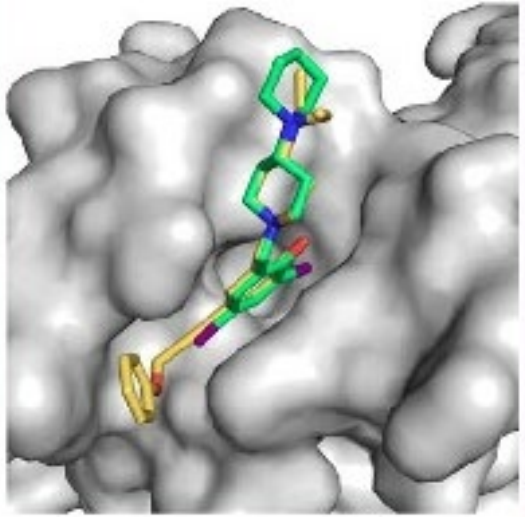
## STRUCTURE-GUIDED DESIGN :




4



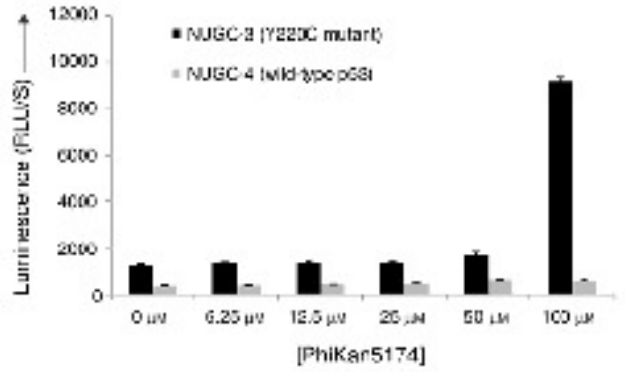



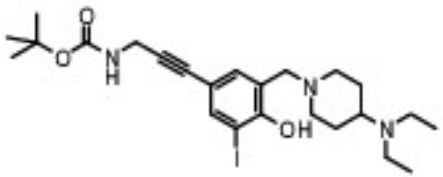



# Halogen Bonds

## APOPTOSIS ASSAY IN NUGC-CELLS:

Caspase-3/7 activities (ApoTox-Glo Triplex assay) at different concentrations of PK5174 and PK5196 in NUGC-3 (p53-Y220C$^{+/+}$) and NUGC-4 (p53-wt$^{+/+}$) cell lines.

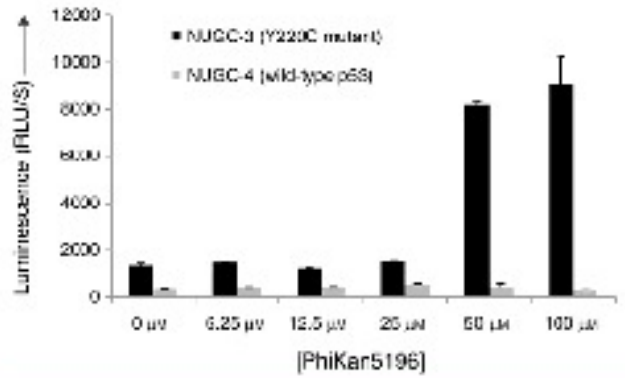


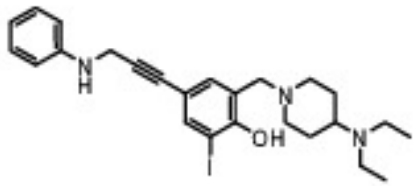




$K_D$ (ITC) = 15,5 µM

$K_D$ (DSF) = 19,7 µM

$\Delta T_M^{app}$ (DSF, 250µM) = 3,2 K




$K_D$ (ITC) = 9,7 µM

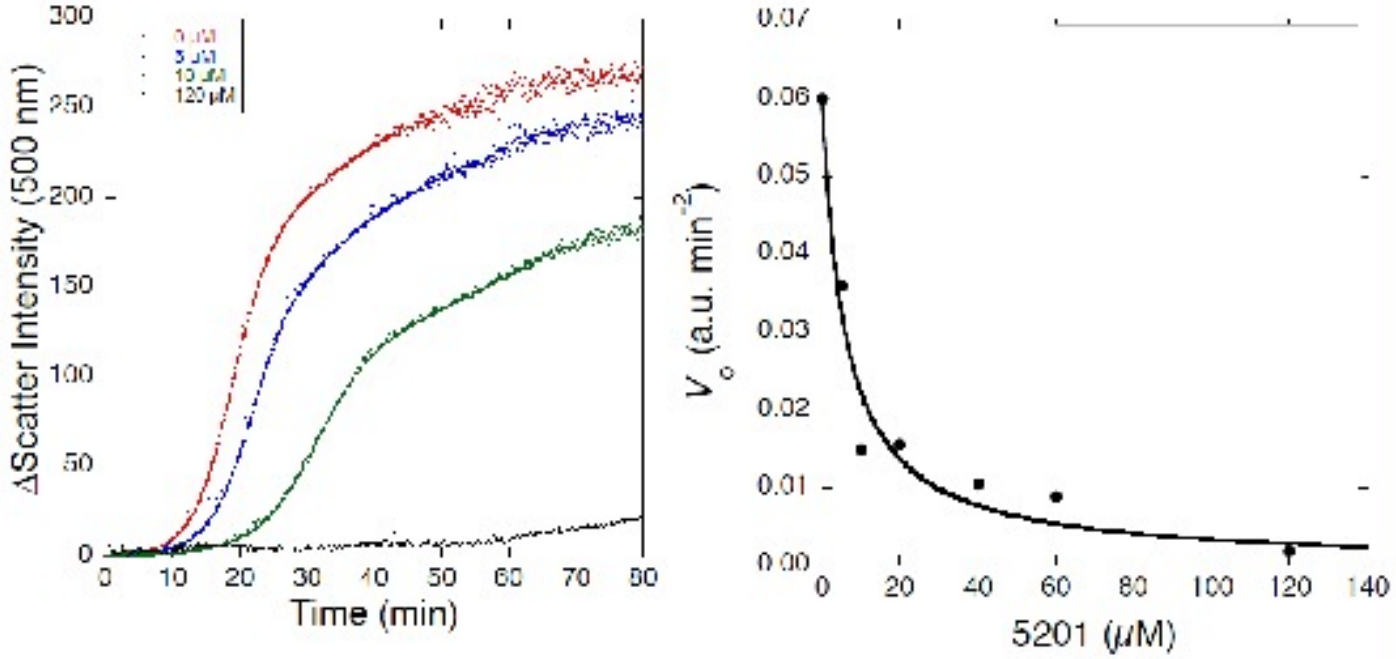
$K_D$ (DSF) = 6,7 µM

$\Delta T_M^{app}$ (DSF, 250µM) = 3,6 K

# Halogen Bonds

## MOLECULAR PROBES: PHIKANS CHANGE P53 AGGREGATION :

Light Scattering at 500 nm indicates concentration-dependent inhibition of p53 aggregation by PK5201

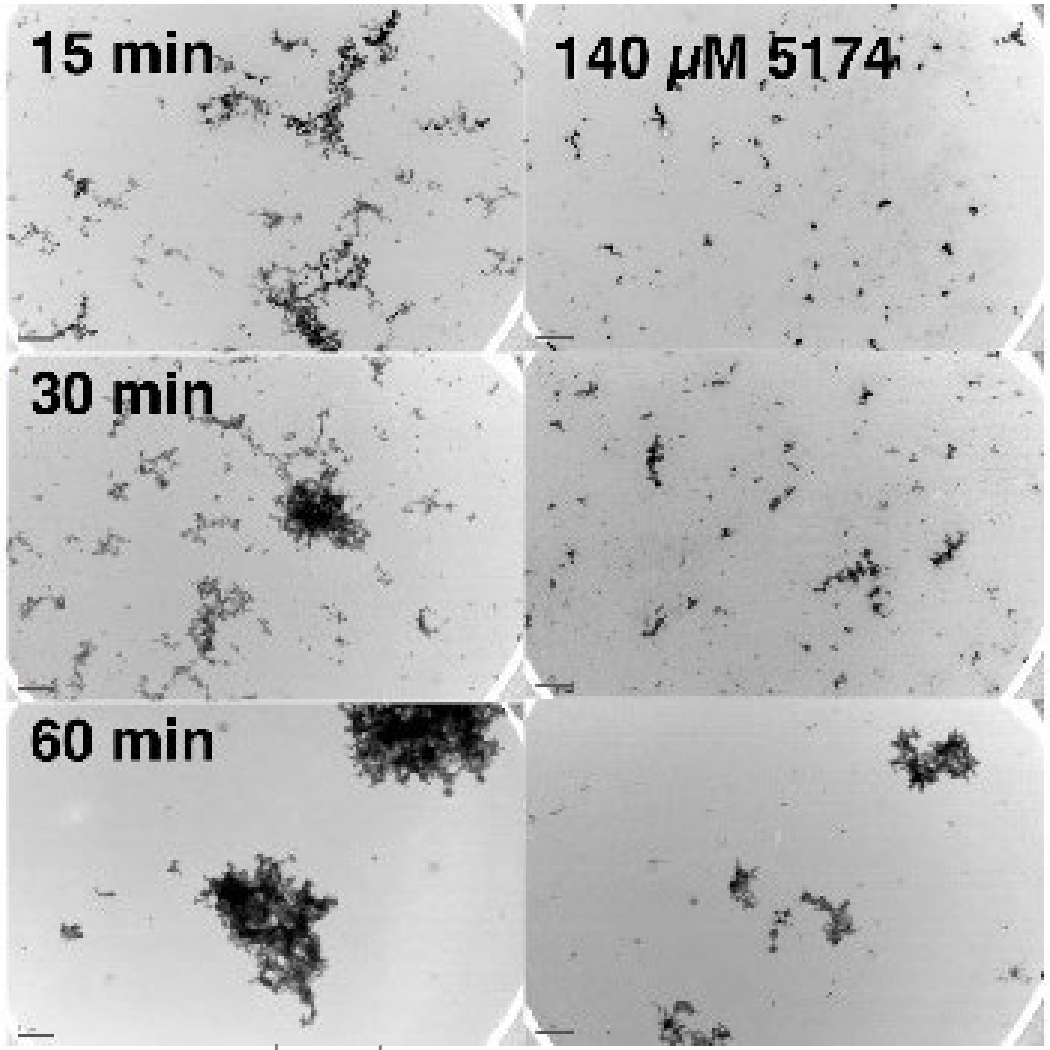



**Wilcken et al., *Proc. Natl. Acad. Sci. U.S.A.* 109, 13584 (2012)**

# Halogen Bonds

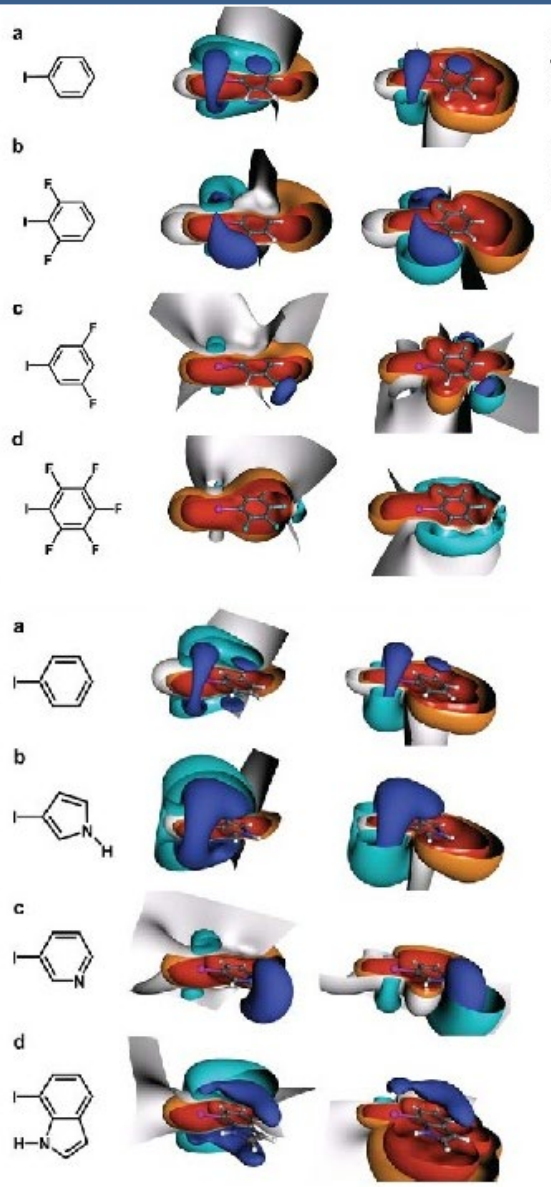
## PHIKANS CHANGE P53 AGGREGATION :

Transmission electron microscopy of time-dependent p53 aggregation in absence or presence of PK5174




| Inhibitor | $K_i$ [μM] $V_0$ (scatter) | $K_i$ [μM] $k_1k_2$ (340nm) | $K_D$ [μM] native state (ITC) |
|---|---|---|---|
| PK083 | 96±3 | 95±30 | 125 |
| PK5174 | 19±4 | 31±4 | 16 |
| PK5176 | 35±4 | 37±7 | 21 |
| PK5196 | 10±1 | 20±4 | 10 |
| PK5201 | 6±1 | 7±3 | 8 |

# Tuning the Strength of Halogen Bond




Substituents can modify the strength of the halogen bond significantly

➜ additional interactions
➜ cooperativity?
➜ binding motifs?

Wilcken *et al.* J. Med. Chem. 56, 1363 (2013)

# Orthogonal Multipolar Interactions vs Halogen Bonds

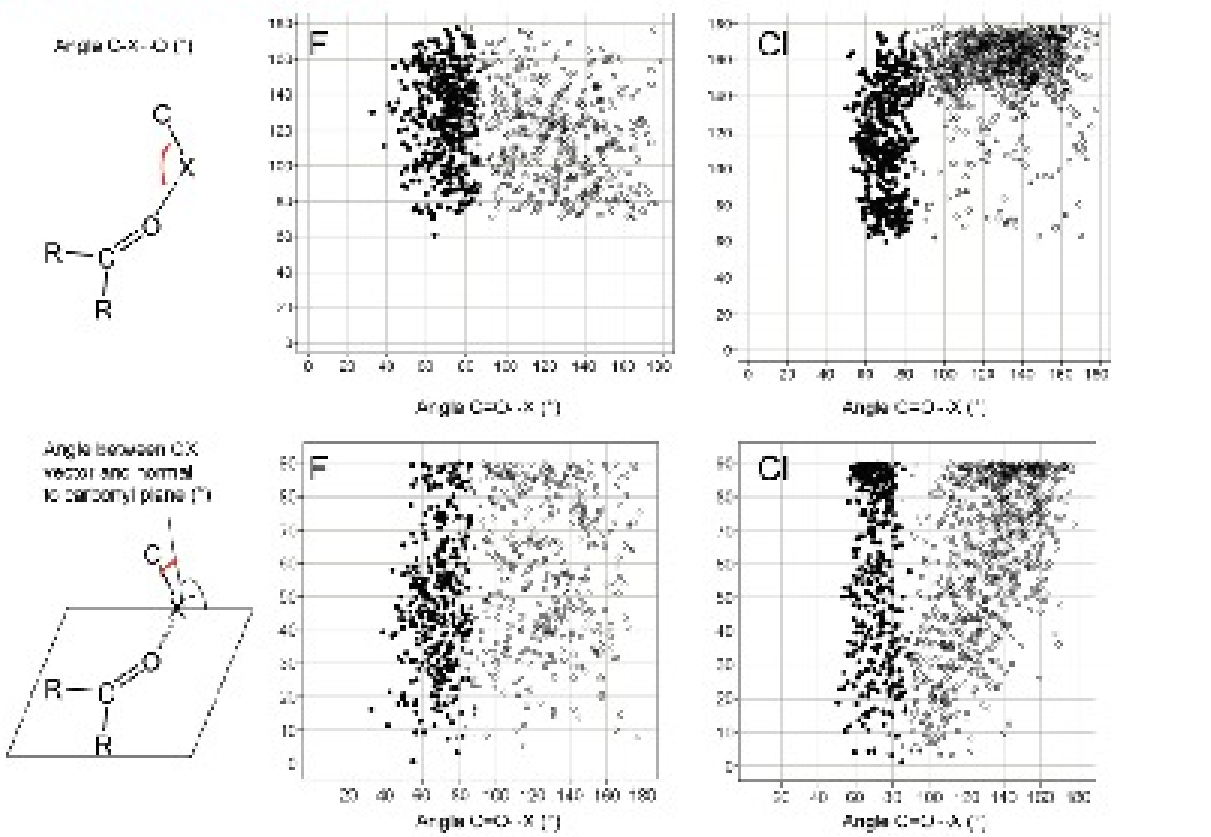


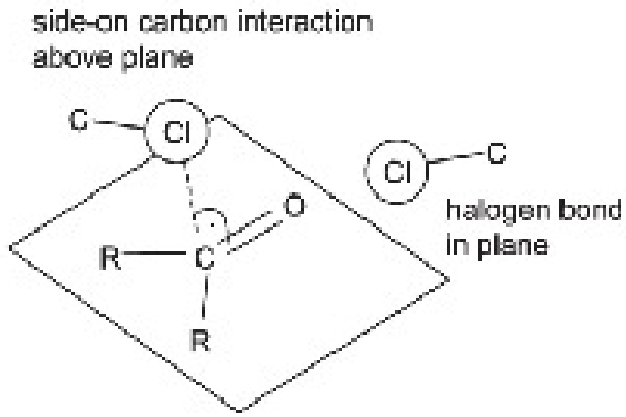

Figure 12. Occurrences of close contacts between F (left) and Cl (right) atoms and carbonyl oxygen in the CSD. Scatter plots show two different angle distributions. Points are categorized by their distance to the carbonyl oxygen atom of the C=O unit (●) close contact between halogen and carbonyl O (< 3.2 Å for F, < 3.5 Å for Cl), (○) close contact between halogen and carbonyl O (< 3.1 Å for F, < 3.3 Å for Cl), (+) both close contact criteria satisfied.

# Halogen Bonds

Some halogens can interact with the oxygen and with the carbon atoms of carbonyl groups ➔ halogen bonds or multipolar interactions

Fluorine shows no orientation preference
➔ distinction between close contacts to carbonyl C and O seems arbitrary!
➔ CF bonds approach carbonyl centers from any direction

Chlorine shows two distinct distributions with a similar frequency:
halogen bonds and multipolar interactions
➔ tendency for the C-Cl bond to be parallel rather than orthogonal to the amide plane (anisotropic distribution of electron density around the Cl atom!)

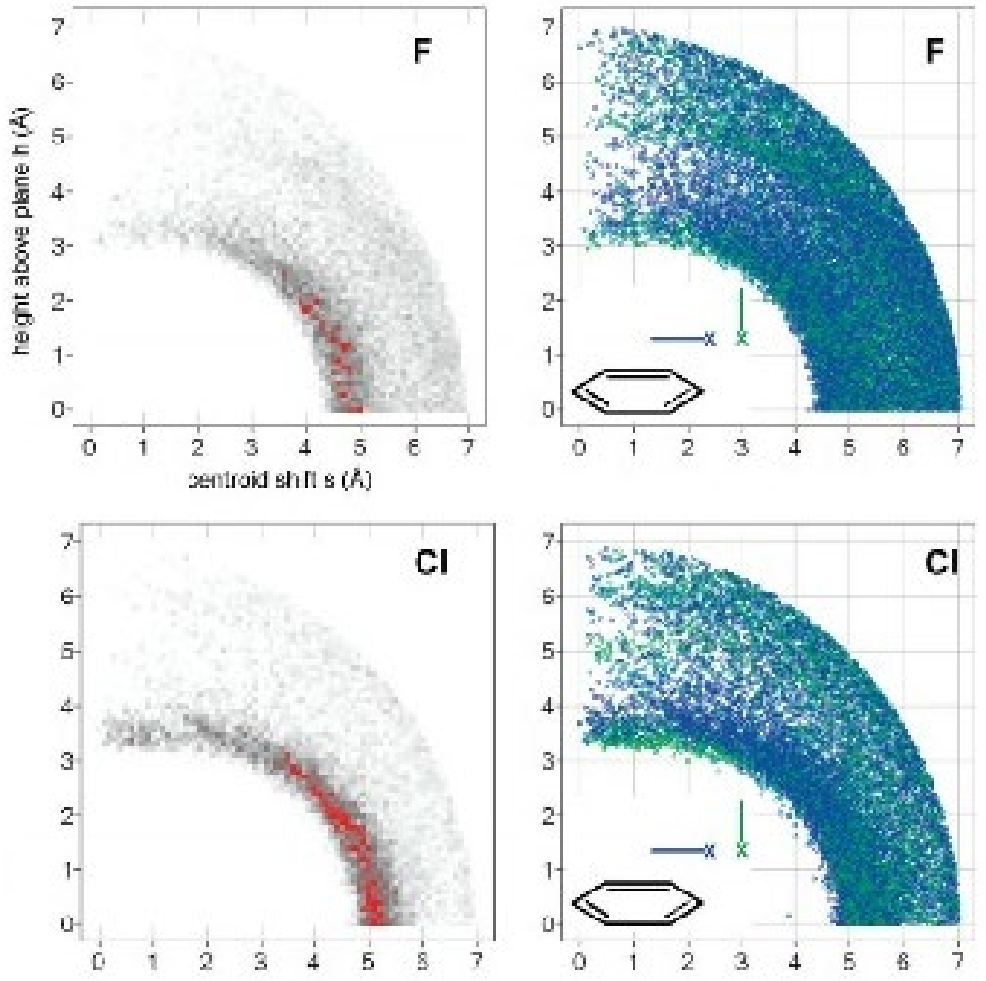



Bromine and iodine:
similar distributions as for chlorine
➔ increasing shift toward more halogen bonds



Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)

# Halogens and Aromatic Rings




Radial distribution of fluorine atoms (top) and chlorine atoms (bottom) around phenyl rings (CSD statistics).

Darker gray corresponds to higher density; peaks above a numerical value of 90 are colored red.
Scatter plots of all hits for fluorine and chlorine (right hand side) are colored by the angle between the phenyl pland and the C-X vector from blue (0°, in plane) to green (90°, orthogonal).



Bissantz *et al.*, J. Med. Chem. 53, 5061 (2010)