Protein interactions with fluorine and other halogens

Anna Vulpetti

Molecular Interactions in Drug Discovery

21 March 2013, Cambridge, UK

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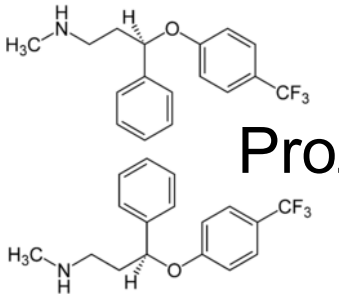
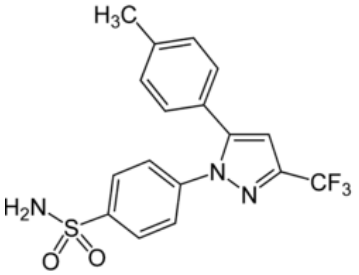
Halogens in Drugs

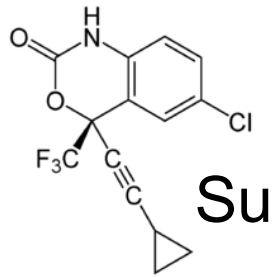


Prozac

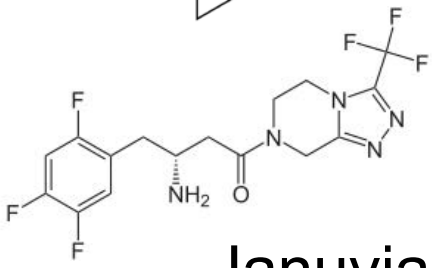


Celebrex

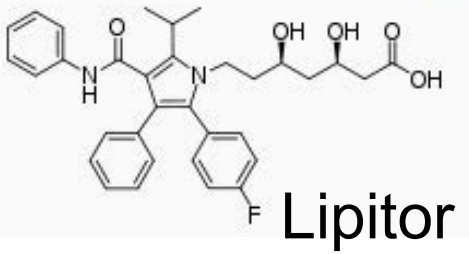
- Fluorine:
  - approximately 20–25% of all drugs contain at least one fluorine atom.
    3 out of 10 best-selling drugs in 2011 contain F.
    7 out of 35 approved drugs in 2011 contain F.
    These include some blockbusters, e.g., Prozac (fluoxetine, depression), Celebrex (celecoxib, arthritis), Sustiva (efavirenz, anti-HIV), Januvia (sitagliptin, diabetes) and Lipitor (atorvastatin, dyslipidemia).



Sustiva



Januvia



Lipitor

- Chlorine, Bromine, Iodine:
  - approximately 14.5% contain Cl, 1.5% Br and 1.2% I of all drugs.

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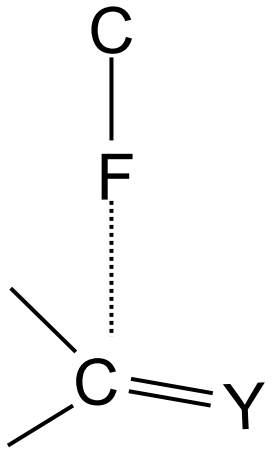
Halogens interactions covered today

- Chlorine, Bromine, Iodine:
  - A **halogen bond** is a non-covalent bond between a halogen atom (X) and a Lewis base (Y).

- Fluorine:
  - **Polar** interaction where Fluorine acts as an acceptor
  - **Hydrophobic** interaction
  - **Multipolar** interaction



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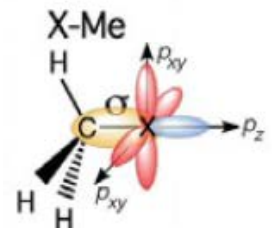
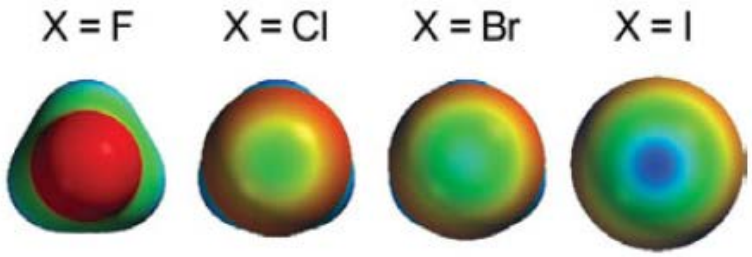
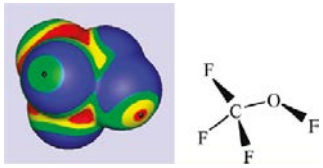
Halogen Bonding and σ-hole: *Principles*

- Halogen bonding was discovered one and a half century ago and its crystallographic description was awarded by the Nobel price in 1969 (O. Hassel).
- The X-bond “donor” is attributed to the anisotropic distribution of the charge density on the halogen atom, resulting from the polarization of the halogen (X) along the C−X σ-bond. According to **molecular orbital theory**, the valence electron in the outer shell p_z orbital participates in the formation of the covalent σ-bond, leaving the orbital depopulated, and thus partially exposing the positive nuclear charge opposite the C-X σ-bond.
- The positive potential on the X is usually referred to as **σ-hole.** This leads to an attractive interaction with **linear arrangement**.
- The **strength** increases as the size of the halogen increases, as the electrons are more polarizable : Cl<Br<I
- This deficiency is compensated by an **electron rich belt around the halogen.**
- **Fluorine** (more electronegative and less polarizable) only forms a σ-hole in very special instances, such as F_2







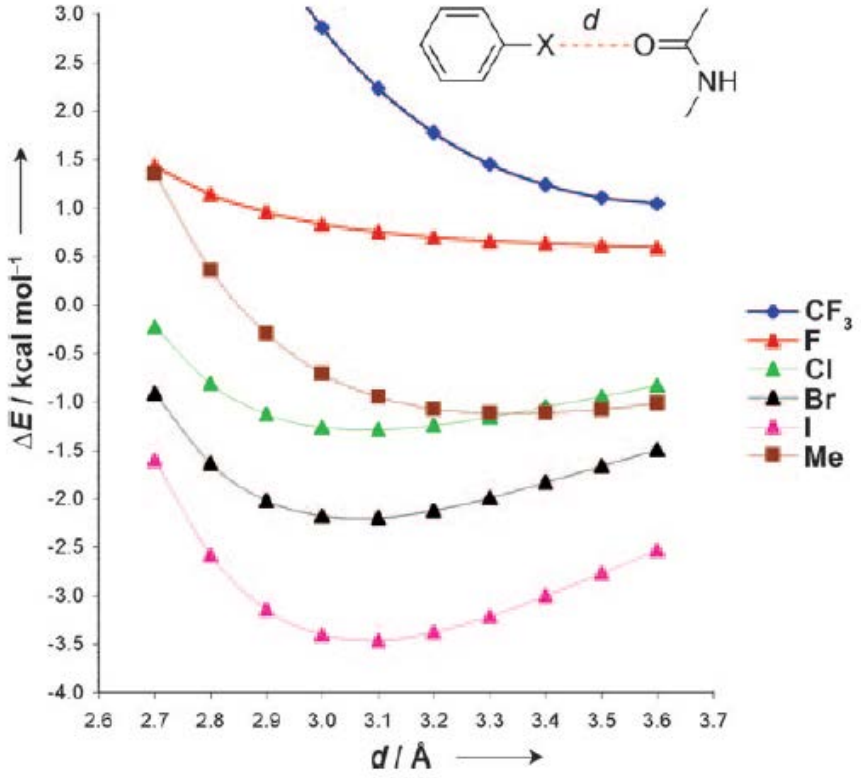
T.Clark et al, J. Mol. Model. 2007, 13(2) 291-296
Lu Y. et al. J.Med.Chem. 2009 May 14;52(9):2854-62

Y.Lu at al. [Review] Expert Opin. Drug Discovery 2012, 7(5) 375-383
R.Wilcken et al. [Perspective] 2013, J. Med. Chem., 1363-1388

O.Hassel, 1970, Science,170, 497

Halogen (Cl,Br,I) Bonding and σ-hole: *Energy*

- Ar-X···O of N-methyl acetamide/acetone



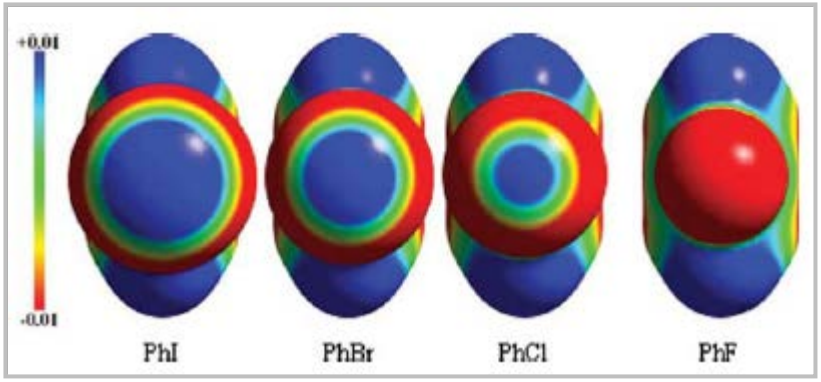
Quantum-based evaluation

Cl···O : 5.4-7.5 KJ/mol

Br···O : 9.0-12.1 KJ/mol

I···O : 14.2-17.6 KJ/mol

H···O : 8.4 KJ/mol



R.Wilcken et al. [Perspective] 2013, J. Med. Chem., 1363-1388

Leo A. Hardegger, et al. Angew. Chem. Int. Ed. 2011, 50, 314 –318

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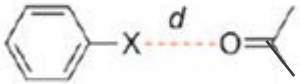
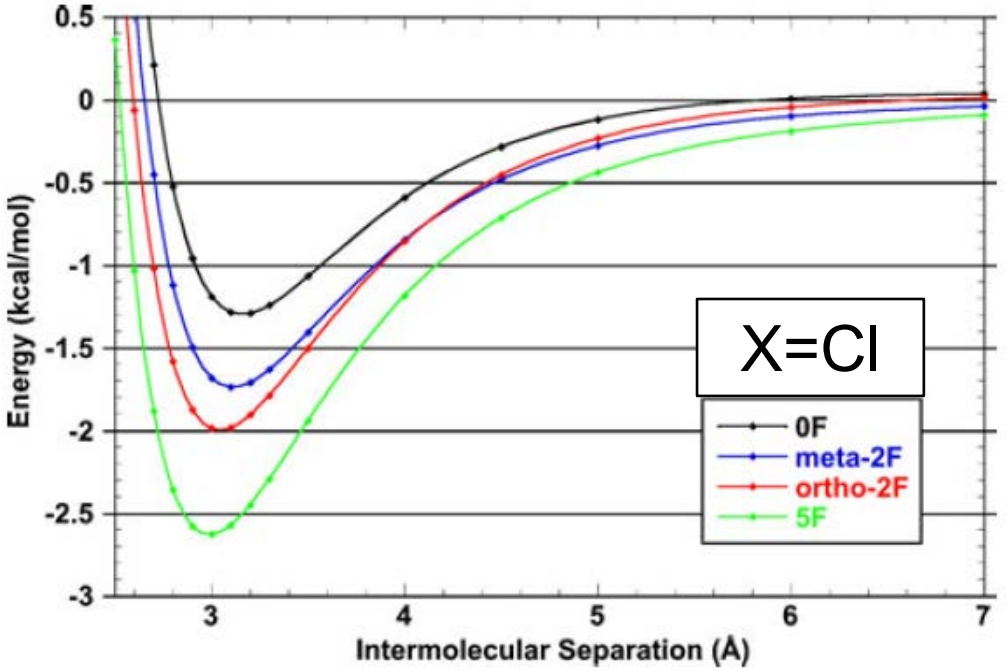
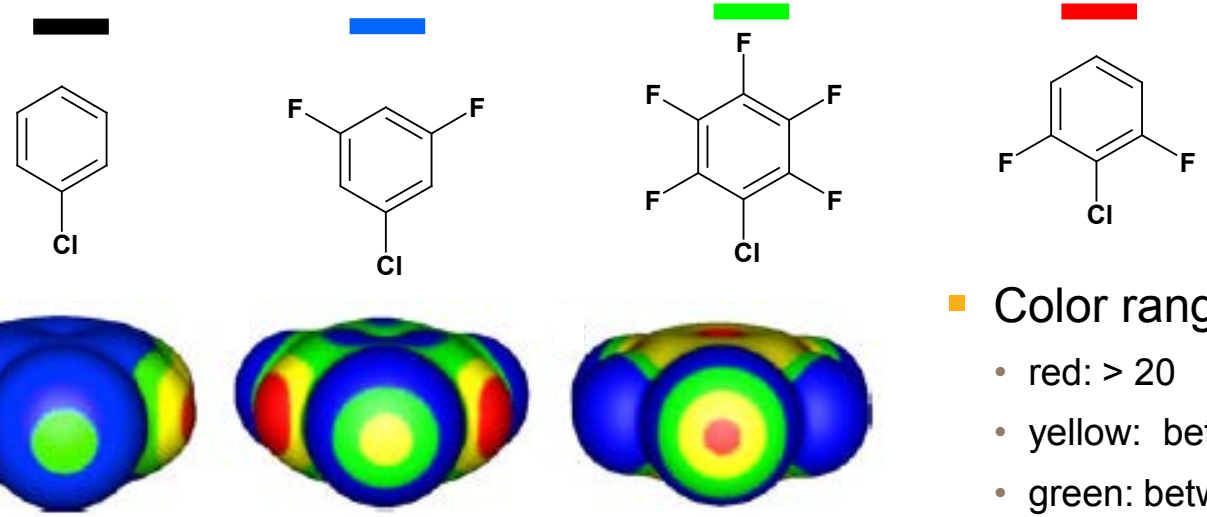
Halogen Bond *Tunability*

Ridley K.R et al. J.Mol.Model. 2011 17(12):3309-3318 (and 2012)







- Color ranges, in kcal mol-1, are:
  - red: > 20
  - yellow: between 20 -10
  - green: between 10 - 0
  - blue: < 0

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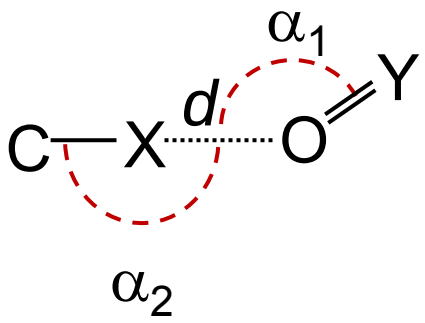
Halogen (Cl,Br,I) Bonding and σ-hole: *Geometry and occurrence* (CSD/PDB)

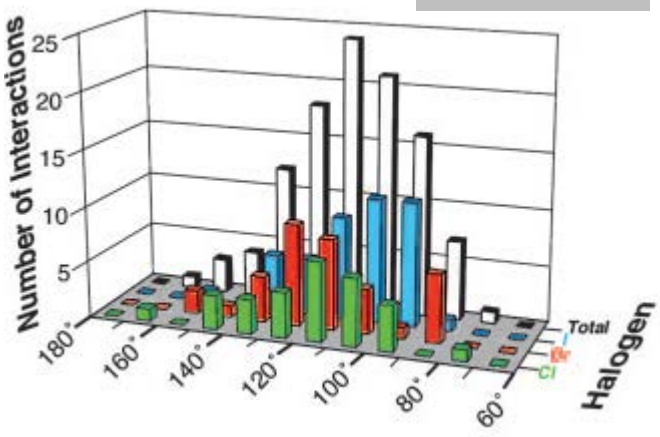


α_1 X...O=C : 90º-180º (120º)
α_2 C-X...O : 140º-180º (165º)

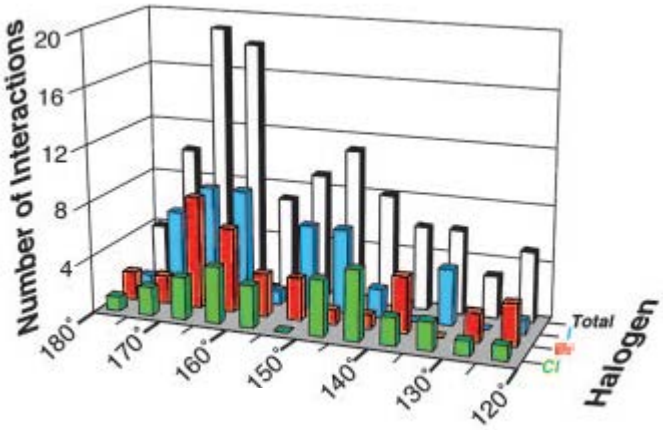
Y= C

X	*d* / Å	% occ. (CSD)	% occ. (PDB)
F	2.99	0.2	1.3
Cl	3.27	2.6	4.9
Br	3.37	4.2	5.8
I	3.5	11.2	14.4

Y= C,P,S



α_1



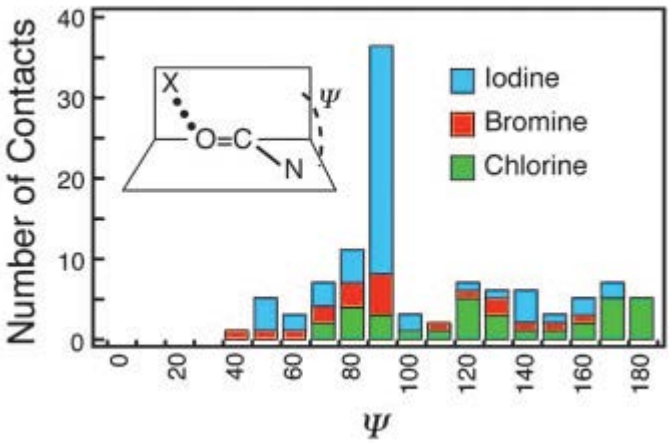
α_2

Leo A. Hardegger, et al. ChemMedChem 2011, 6, 2048–2054

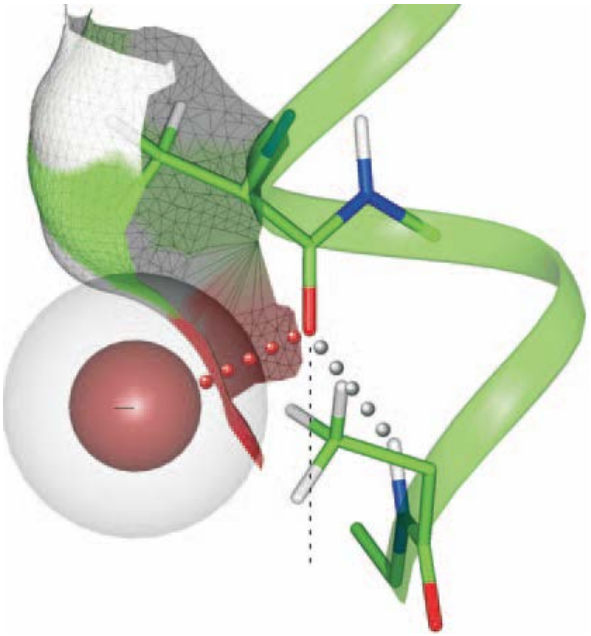
Auffinger, et al. PNAS, 2004, 101(48), 16789–16794

Halogen bonds as Orthogonal molecular interactions to H-bonds

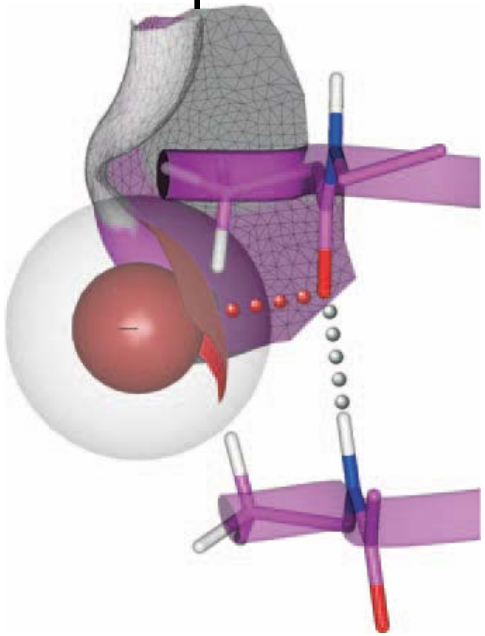
- Halogen-bonds are also observed perpendicular to the oxygen of peptidic C=O involved in α-helices and β-sheets.
- Halogenated compound can form an halogen-bond to a carbonyl oxygen atom that is already H-bonded.



α-helix



β-sheet

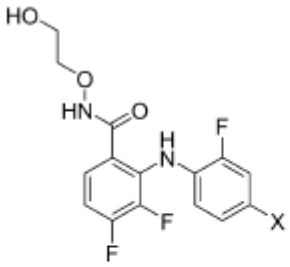


Voth, A.R. et al., Nature Chemistry, 2009, 1, 74-79

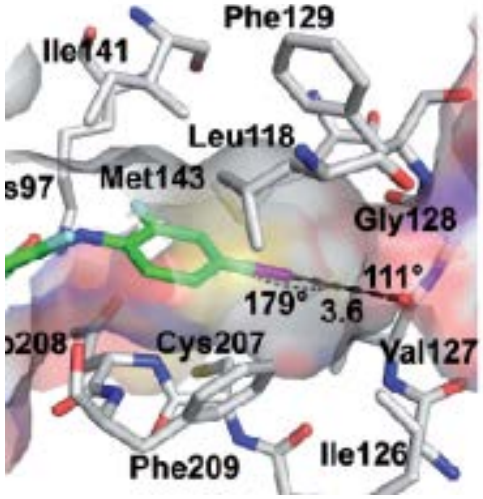
Halogen (Cl,Br,I) Bonding and σ-hole: *systematic experimental evaluation*

Leo A. Hardegger, et al. ChemMedChem 2011, 6, 2048 – 2054 (MEK1, hCatL)

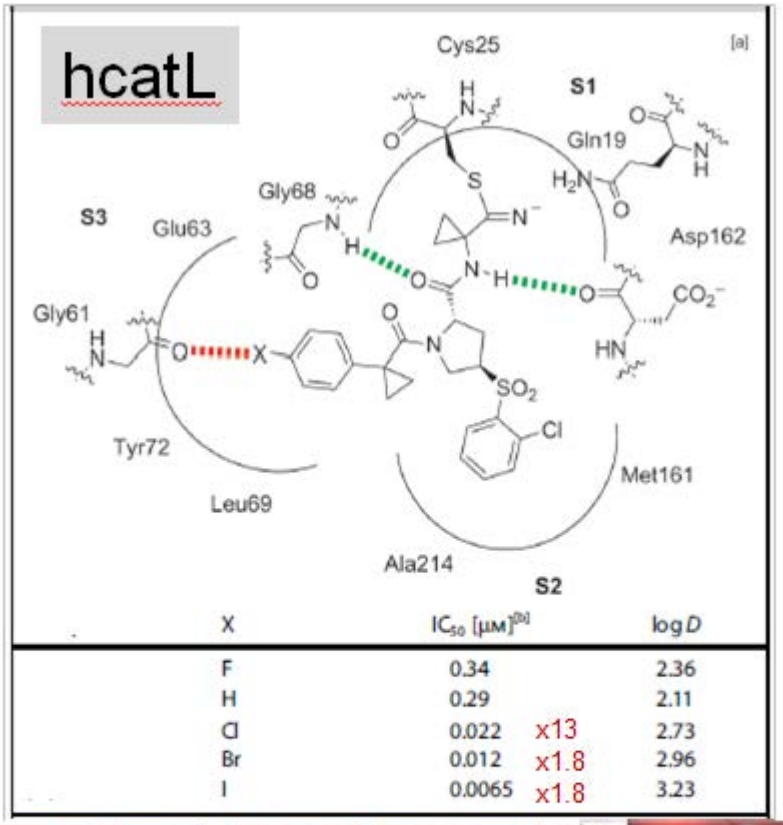
MEK1



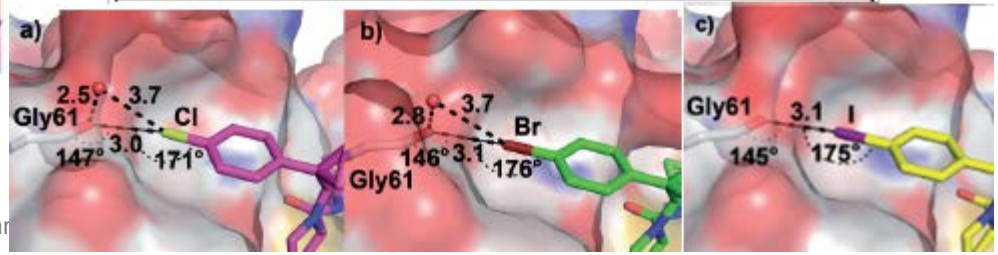
X		IC_{50} [μM] (MEK)
F		0.12
H		0.052
Cl	x2	0.026
Br	x3.4	0.0077
I	x3.8	0.0020



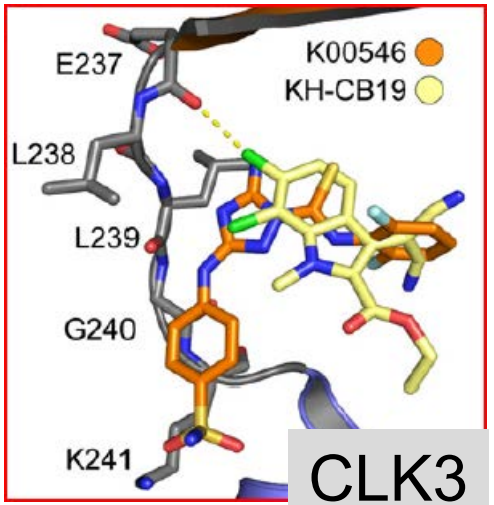
hcatL



X	IC_{50} [μM][b]		log *D*
F	0.34		2.36
H	0.29		2.11
Cl	0.022	x13	2.73
Br	0.012	x1.8	2.96
I	0.0065	x1.8	3.23

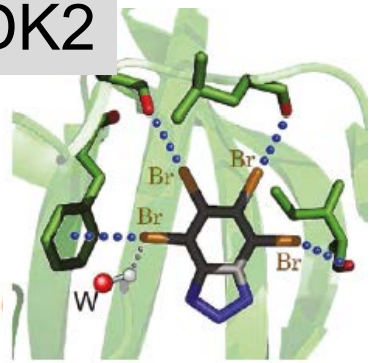


Fedorov O. et al. Chemistry & Biology 2011, 18, 67–76 (CLK3)



CLK3

CDK2



Protein interactions with fluorine

- **Polar Interaction with H-bond donors**
  - NH backbone, Polar side chain (His, Ser/Thr/Tyr, Asn/Gln)
    aromatic C-H (e.g., Phe/Tyr)
    polarized Cα–H
    and protein bound water
- **Hydrophobic Interaction with lipophilic side chains**
- **Multipolar interaction with**
  - C-F... C=O backbone and amide side-chain (Asn/Gln)
  - C-F... guanidinium of Arg

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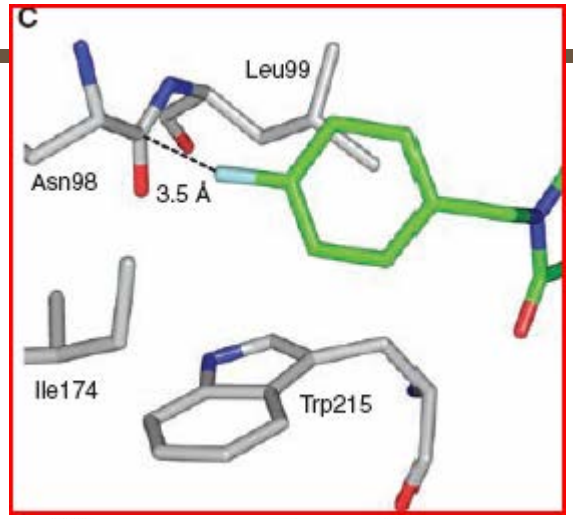
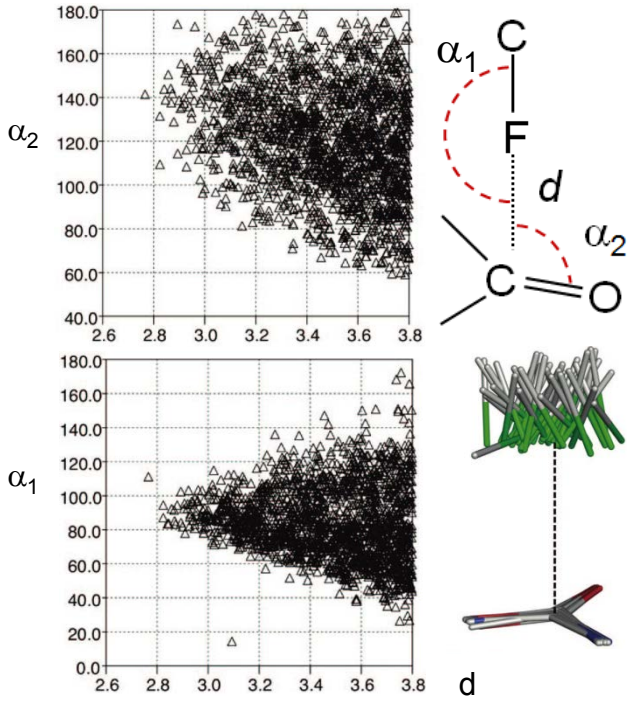
Müller et al. Science 2007 , 317, 1881
Zurcher M, Diederich F. J. Org. Chem. 2008, 73, 4345–4361

Multipolar interaction

- Orthogonal multipolar C–F···C=O interactions were nicely revealed during the fluorine scan of tricyclic thrombin inhibitors.
- Introduction of F in the para position of the benzyl ring occupying the D pocket of thrombin enhanced the binding affinity by a factor of 6 (ΔΔG = –1.1 kcal mol−1, i.e. the difference in binding free enthalpy between para- F-substituted and unsubstituted ligands).
- X-ray crystallography revealed that the C–F residue interacts in an orthogonal fashion, with the backbone C=O group of Asn98 and it is at short distance with H–Cα, with a F···C distance of 3.1 Å.
- Such orthogonal multipolar interactions were subsequently shown to be abundant in both small molecule x-ray crystal structures (CSD) and in protein-ligand complexes (PDB).





LEF and Rule of Shielding

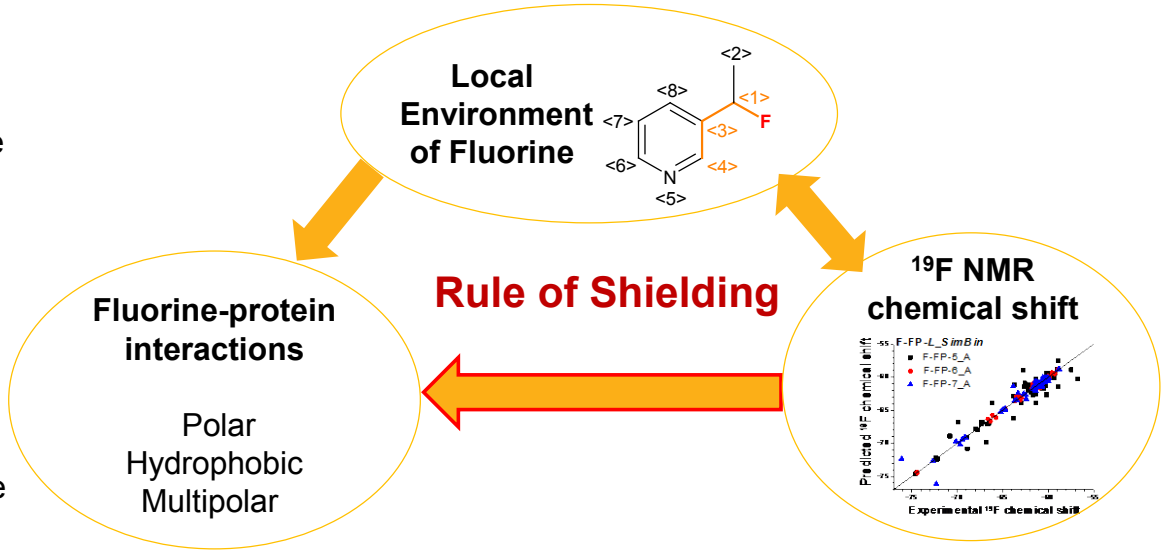
The amphiphilic character of Fluorine, as a hydrogen bond acceptor and hydrophobic moiety, can be correlated to the ^{19}F NMR chemical shift

The character to the C-F bond depends on the **Local Environment of the Fluorine** (**LEF**).

In 2009 we hypothesized that the LEF, which determines the **^{19}F-NMR chemical shift**, might be relevant for the type and nature of molecular interactions with the protein.

In fact, the ^{19}F-NMR chemical shift of the F is affected by a different electronic charge distribution around F

The hypothesis was later supported in 2011 by the analysis of the correlation between the fluorine chemical shift and the type of fluorine-protein interactions observed in crystal structures (“**Rule of Shielding”**)



Vulpetti, A.; Dalvit, C. *Drug DiscoveryToday* **2012**, 17, 890-897

Vulpetti, A., Hommel, U., Landrum, G., Lewis, R., Dalvit, C., J. Am. Chem. Soc. 131 (2009) 12949-12959

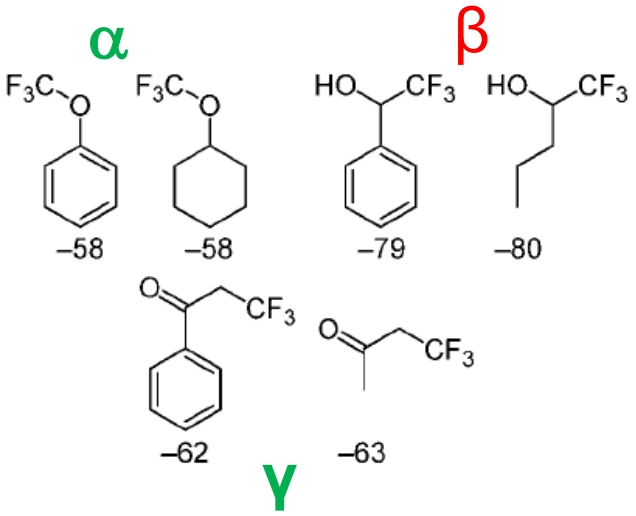
Dalvit C. ,Vulpetti A. ChemMedChem., (2011), 6, 104-114

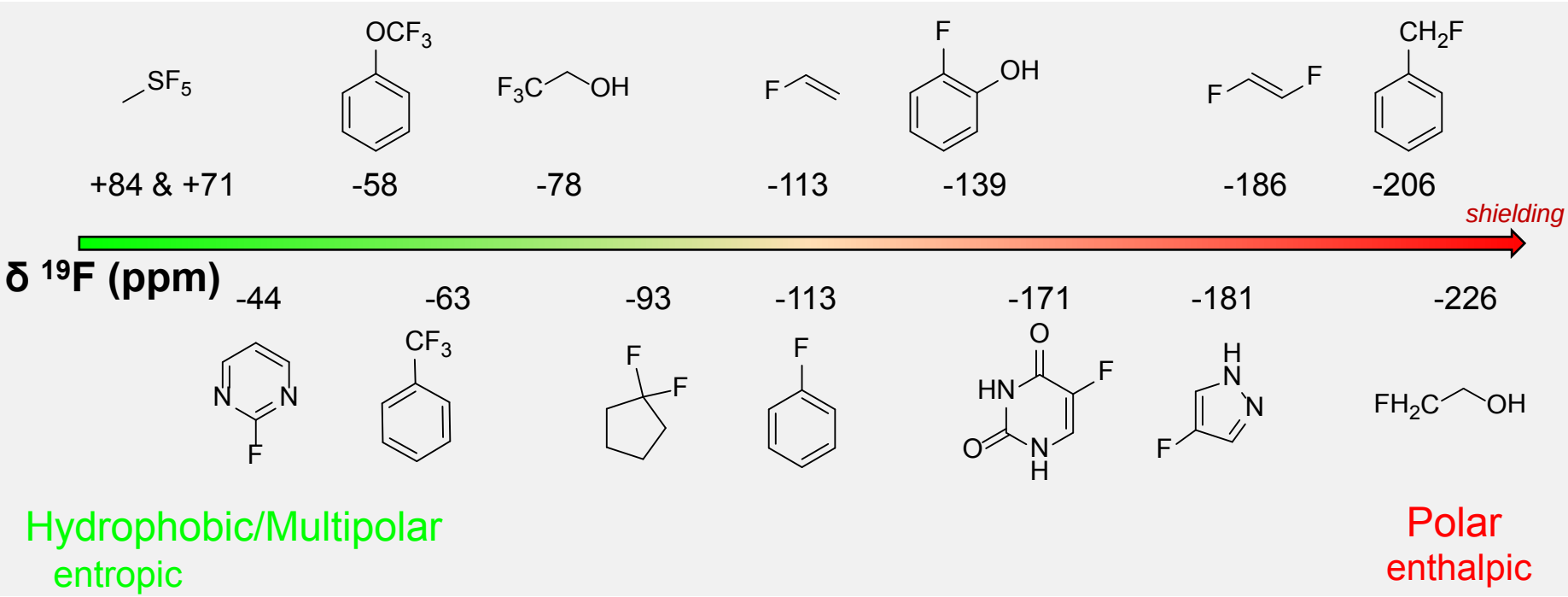
Dalvit C.,Vulpetti A. ChemMedChem., (2012), 7, 262-272

Dalvit C., Ko, S.Y., Vulpetti A. J.Fluorine Chemistry, (2013), in press, DOI:10.1016/j.bbr.2011.03.031

^{19}F NMR Chemical Shifts range

- Large dispersion in ^{19}F-NMR chemical shifts (> 300 ppm)
- Fluorine chemical shifts for CF, CF_2, and CF_3 groups are strongly modulated by the number of oxygen, sulfur, nitrogen, and halogen atoms and their positions (α, β and γ) with respect to fluorine





Hydrophobic/Multipolar
entropic

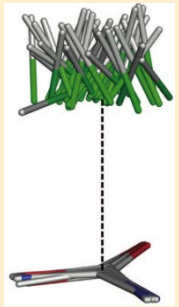
Polar
enthalpic

Fluorine-protein interaction searching

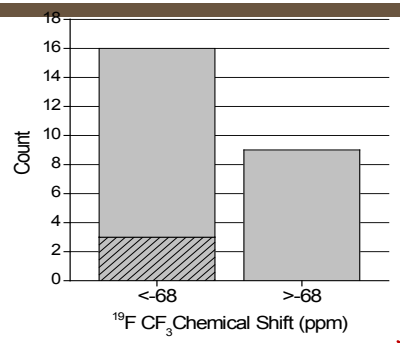
Hydrophobic / Multipolar Contacts *in the* ***PDB***

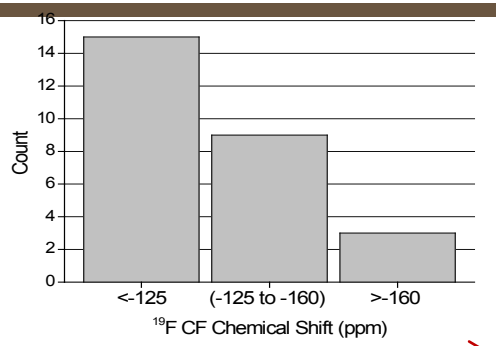
F···C (CD1,CD2 Leu)

F···C=O Backbone



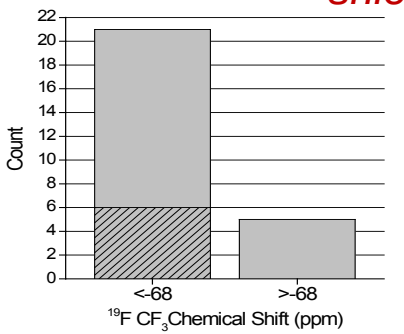
F···S (Methionine)

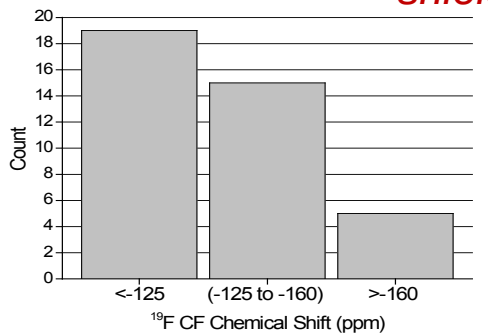


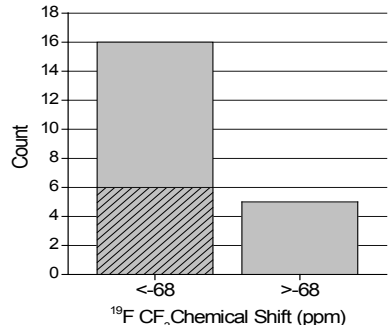


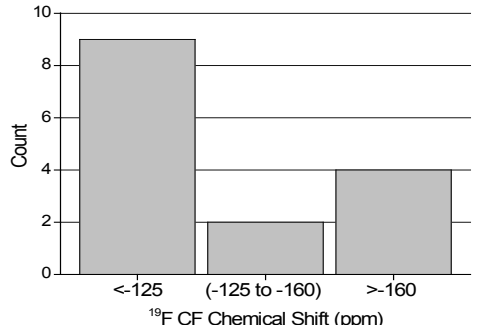
shielding

shielding









Deshielded fluorines are found preferentially in close contact with ***hydrophobic side-chains*** and with the ***carbon of the carbonyl***.

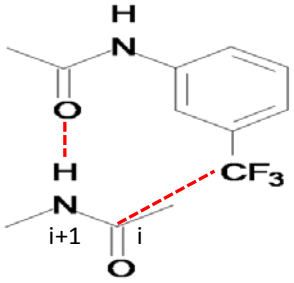
Motifs containing deshielded CF_3

Recognition of different protein structural motifs

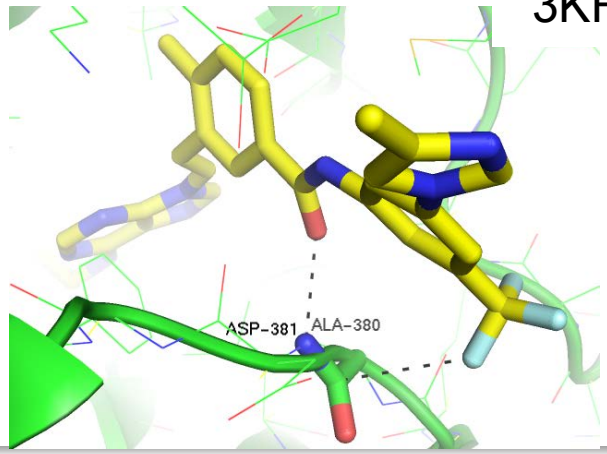
Extended conformation

3KFA

Ligand

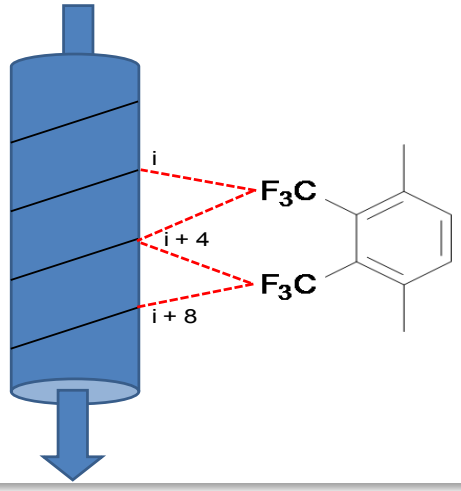


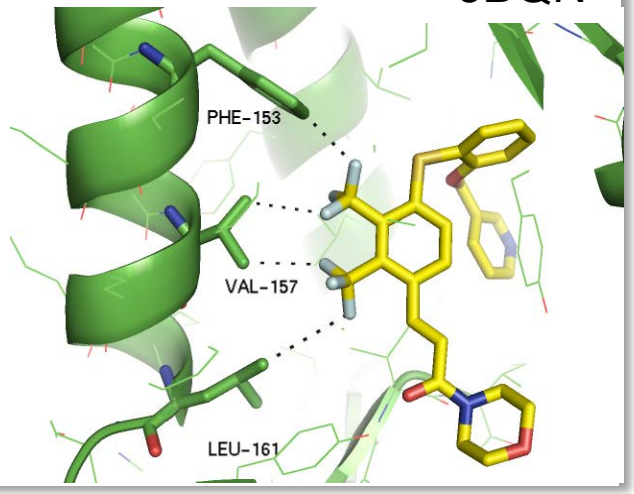
Protein



α-Helix conformation

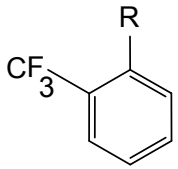
3BQN

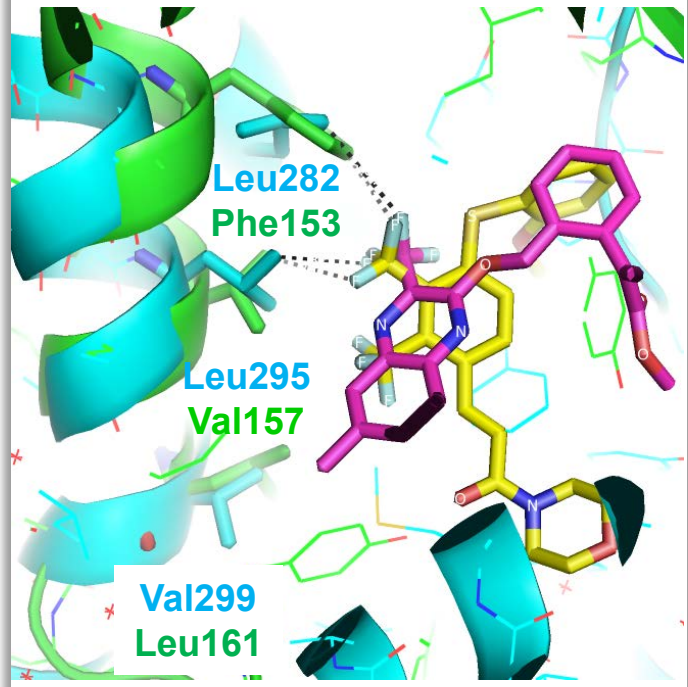




Dalvit C. ,Vulpetti A. ChemMedChem., (2011), 6, 104-114

Similar hydrophobic protein environment





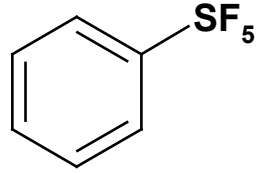
3BQN (green/yellow): same helix

3TGU (cyan/purple): 2 helixes

Dalvit C., Ko S.Y, Vulpetti A. J. Fluorine Chem., (2013), in press

Deshielded Pentafluorosulfanyl fluorines (SF_5)

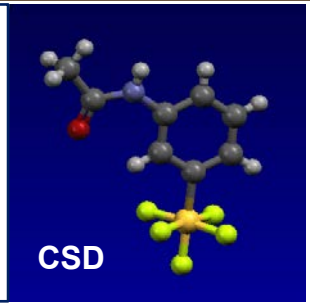
- SF_5 (orange) smaller than a *t*-Bu larger than a CF_3 similar to OCF_3

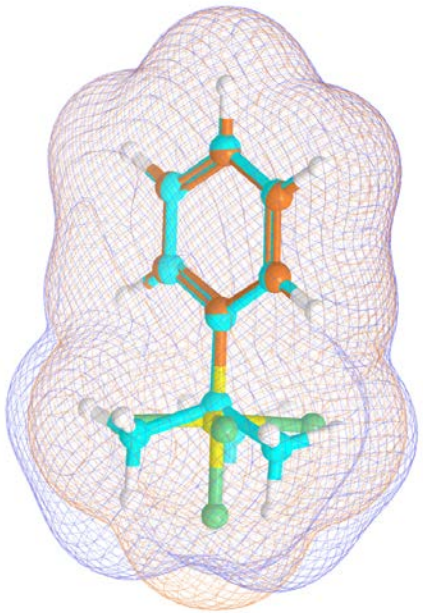


19F-NMR

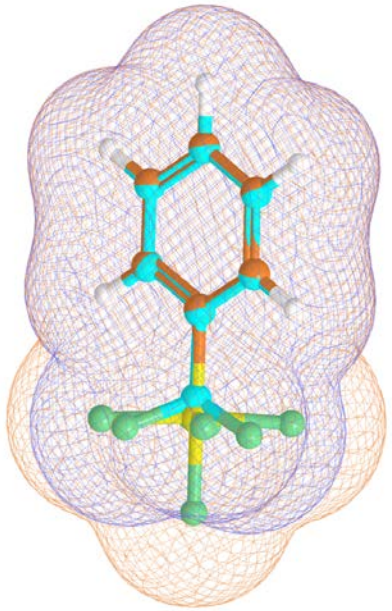
+62 ppm (doublet, 4F, eq)
+84 ppm (pentet, 1F, ax)

$^2J_{FF}$ = 149 Hz

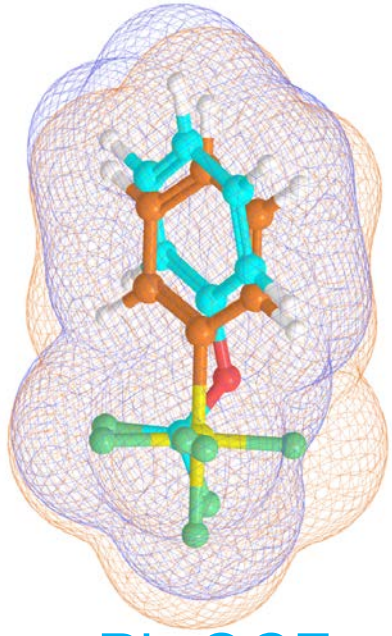




Ph-$C(CH_3)_3$



Ph-CF_3



Ph-OCF_3

Dalvit C., Ko, S.Y., Vulpetti A. J. Fluorine Chemistry, (2013), in press, DOI:10.1016/j.bbr.2011.03.031

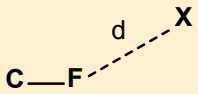
Fluorine-protein interaction searching

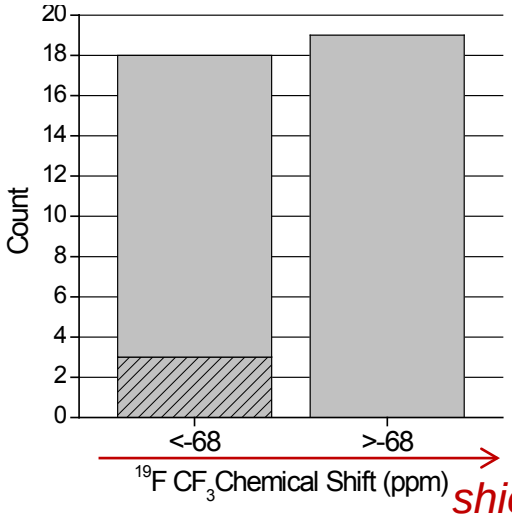
Polar Contacts - *Close F···N (Sidechain) Contacts in the **PDB***

Shielded fluorines, i.e., with increased electron density, are observed preferentially in close contact to ***hydrogen bond donors*** of the protein suggesting the possibility of intermolecular hydrogen bond formation.

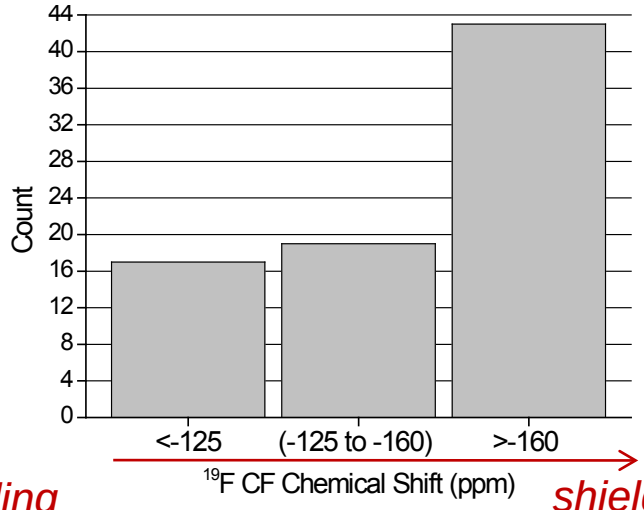
All Contacts

F··· N ≤3.2 Å





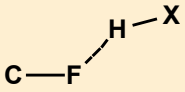
shielding

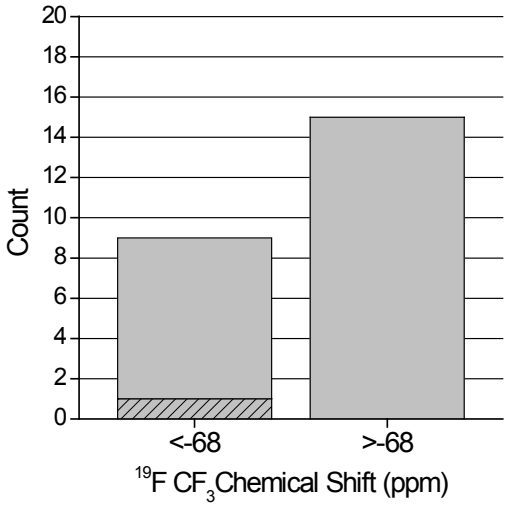


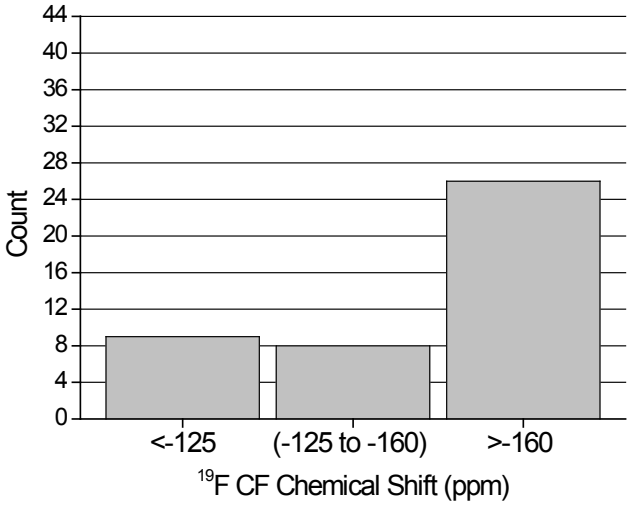
shielding

α_1

110°≤ F···H-N ≤180°



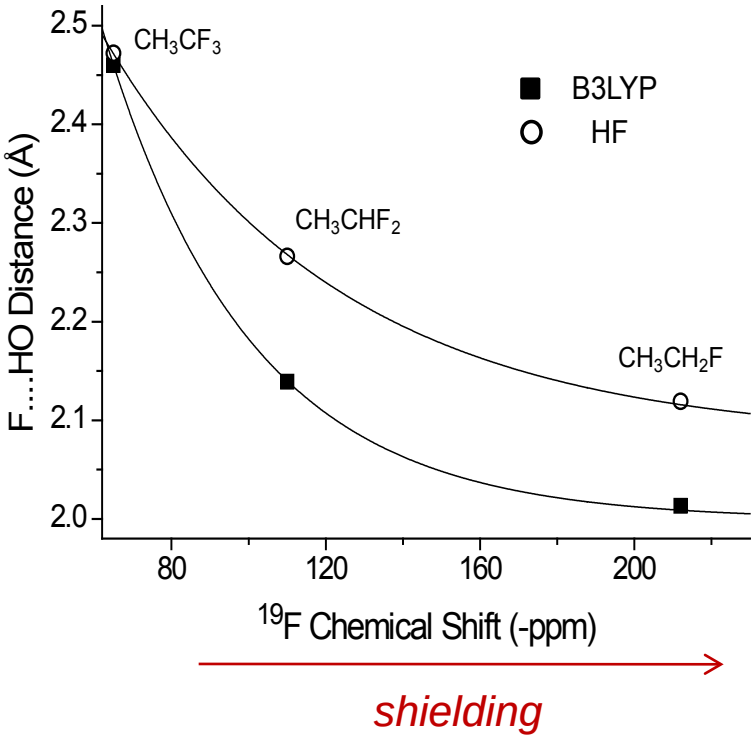




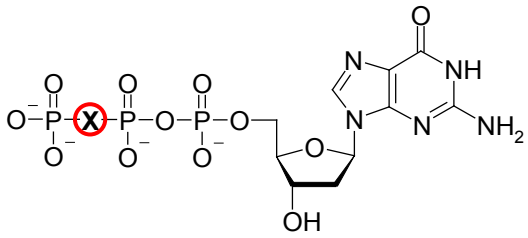
Polar Contacts

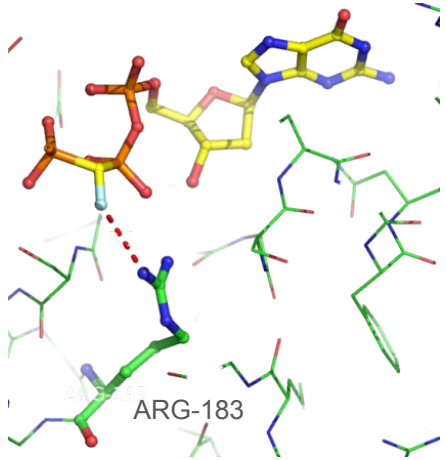
F...H-X (X=O,N) ***calc./exp.*** *distance vs.^{19}F NMR Chemical Shifts*

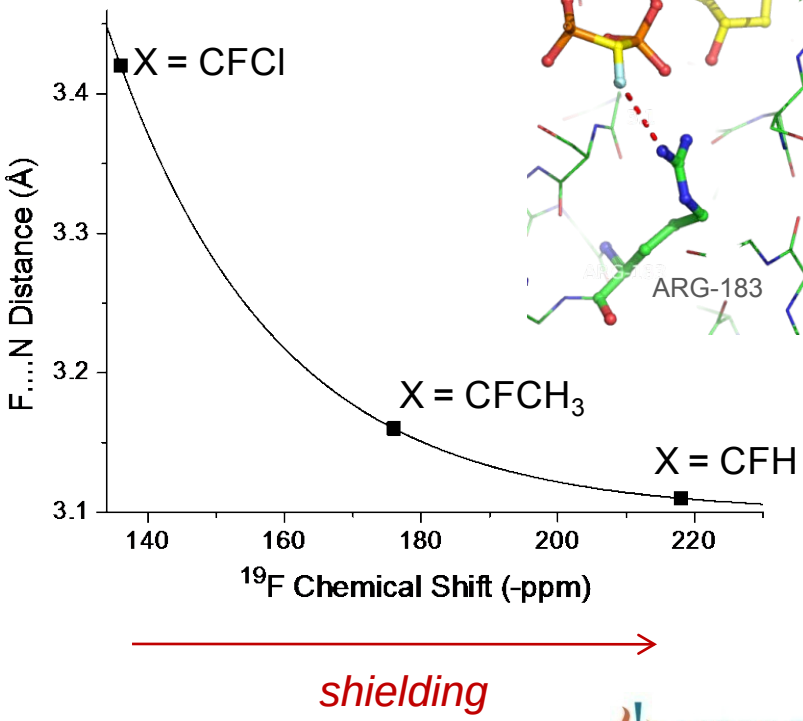
- **Ab initio** F…HO of EtOH distance [HF and B3LYP (6-311G**++)]



- **Experimental** F...N of NH_2 Arg183 in the X-ray structures of DNA polymerase β with a series of β,γ-X dGTP analogues







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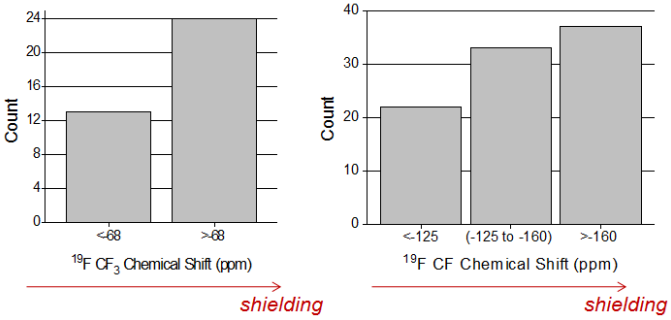
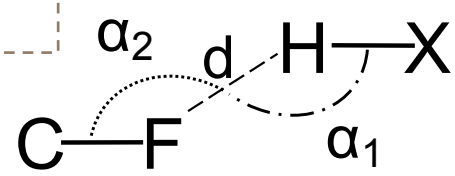
Intermolecular short contacts

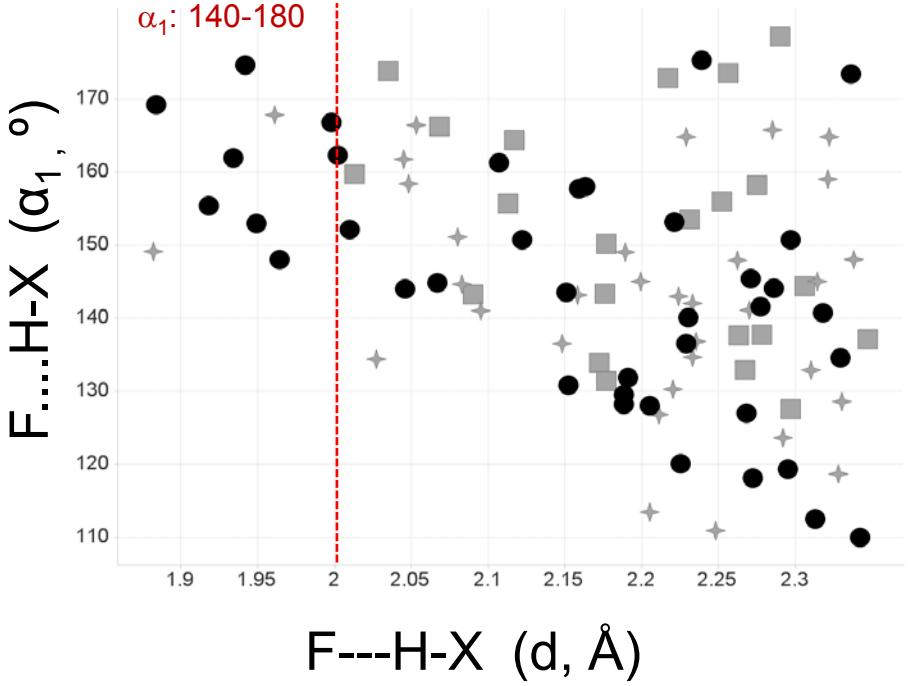
F---H-X (with X=O, N) in the **CSD**

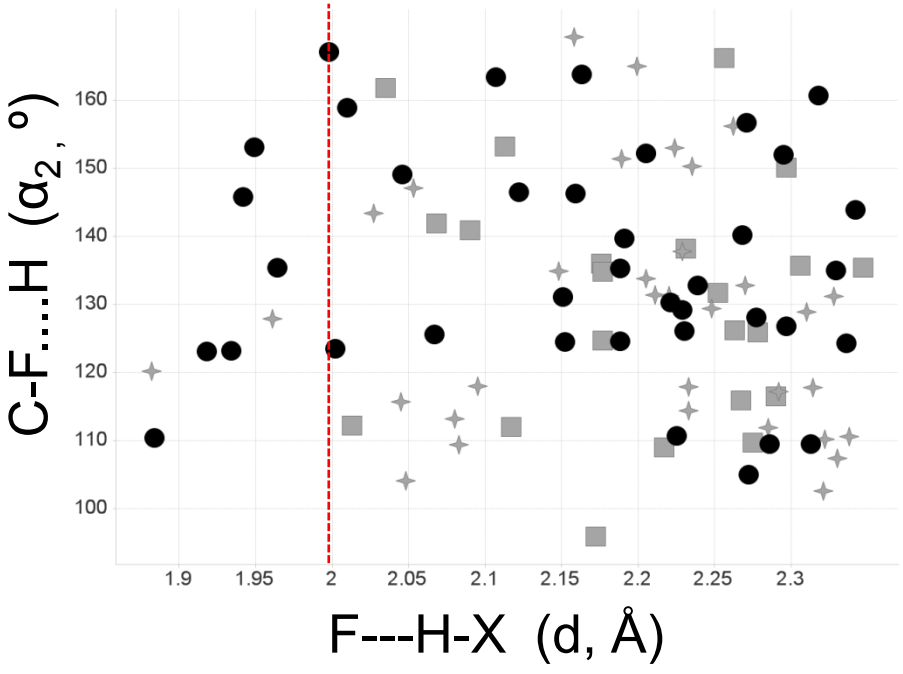


Intermediate shielding -125 ppm to -160 ppm
Least shielded <-125 ppm
Most shielded >-160 ppm



d (F---H) < 2 Å
[shielded]
α_1: 140-180





Dalvit C., Vulpetti A. ChemMedChem., (2012), 7, 262-272

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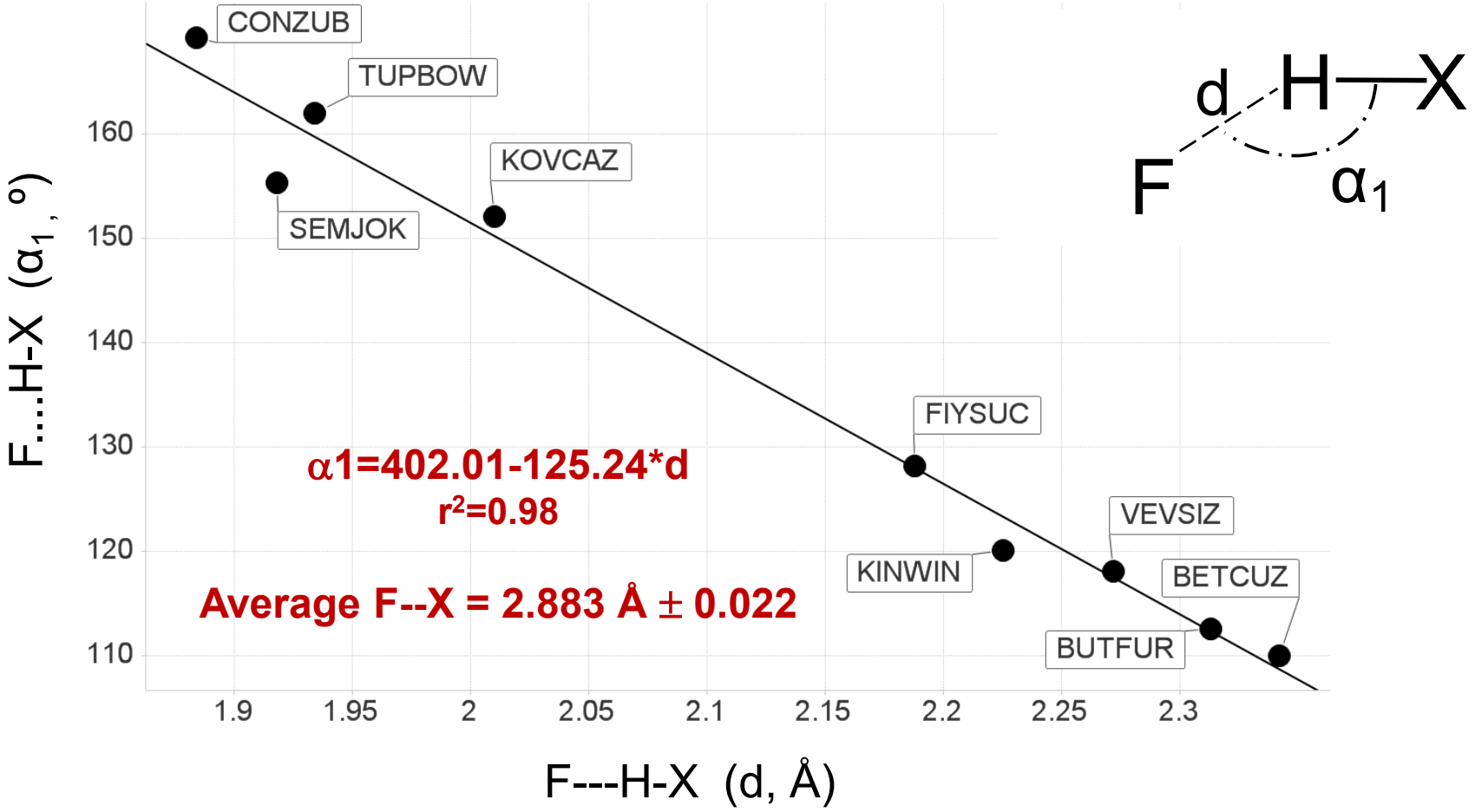
Correlation between α1 and d

Primary (R-CH_2F), shielded secondary alkyl fluorines (R'R''-CHF)



Dalvit C., Vulpetti A. ChemMedChem., (2012), 7, 262-272

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LEF & Rule of shielding
Application in Fragment based screening

Based on these findings we encoded the description of the **Local Environment around Fluorine** in a **novel fingerprints descriptor** (**F-FP-L**), which was used to :

- design a novel Novartis diverse **fragment library of fluorinated compounds** for **^{19}F NMR-based screening,** known as **LEF library(ies)**
  - by selecting fragments from commercial/in-house collections
  - by Diversity-Oriented Synthesis (LEF-DOS) (enhanced 3D-shape)
- design **mixture composition** with no overlap in ^{19}F NMR chemical shift

*Vulpetti, A.; Dalvit, C. Drug DiscoveryToday (*2012), 17, 890-897

Vulpetti, A., Hommel, U., Landrum, G., Lewis, R., Dalvit, C., J. Am. Chem. Soc. 131 (2009) 12949-12959

Vulpetti, A., Landrum, G., Rüdisser, S., Erbel, P., Dalvit, C., J. of Fluorine Chem., 131 (2010) 570-577

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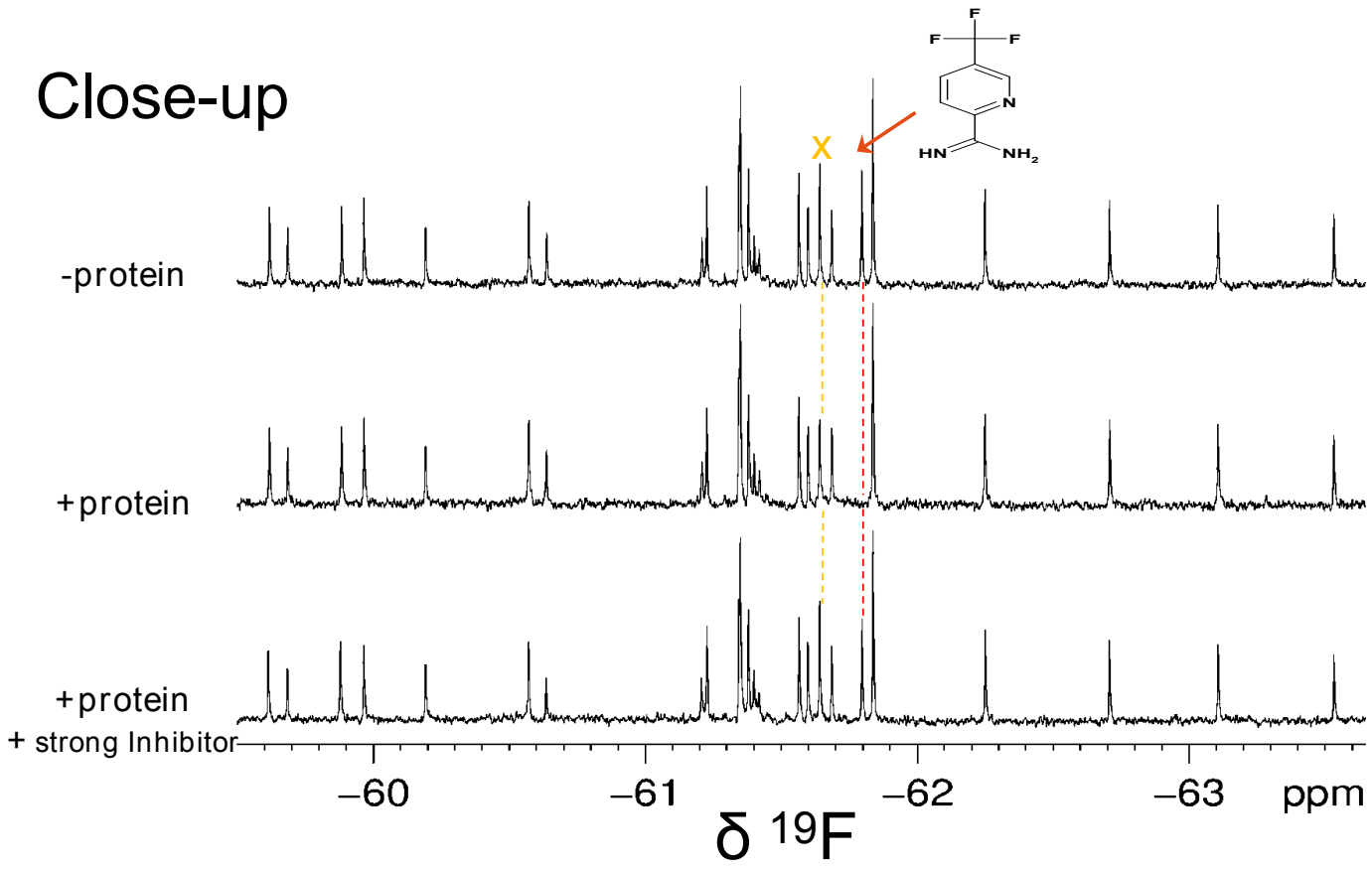
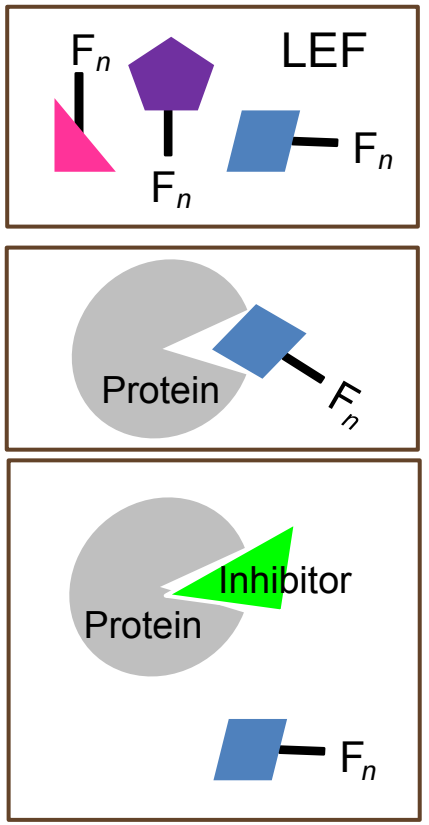
^{19}F-NMR Screening with a **Large Mixtures** (36 Molecules) and **Validation of the Hits** via Competition Binding Experiments





Typical conditions: 0.5-5 μM protein /15 μM (CF_3), 35 μM (CF) ligand /mixture (15 to 36)

Vulpetti, A., Hommel, U., Landrum, G., Lewis, R., Dalvit, C., J. Am. Chem. Soc. 131 (2009) 12949-12959

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Summary

- Large number of ligands have halogens incorporated in their structure
- Halogens have an anisotropic charge distribution with an equatorial ring of negative charge and a region of positive charge along the C-X bond (σ-hole)
- Larger halogens form larger (more positive) σ-hole. The size of the σ-hole is affected by the electronegativity of the atoms near the C-X bond
- Large majority of halogen bond acceptor are carbonyl moieties in PDB
- Fluorine behaves different from other halogens
- Not all Fluorines are identical, the Fluorine local environment is relevant for the preferred protein-fluorine interaction
- The amphiphilic character of fluorine, as a hydrogen bond acceptor and a hydrophobic moiety, can be correlated to the ^{19}F chemical shift
- The derived "Rule of Shielding" provides insight and guidelines in the field of lead optimization and in the design of novel fluorinated chemical scaffolds that recognize distinct protein structural motifs

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Acknowledgments

Claudio Dalvit *(University of Neuchâtel)*

Paul Erbel, Simon Rüdisser

Greg Landrum, Richard Lewis

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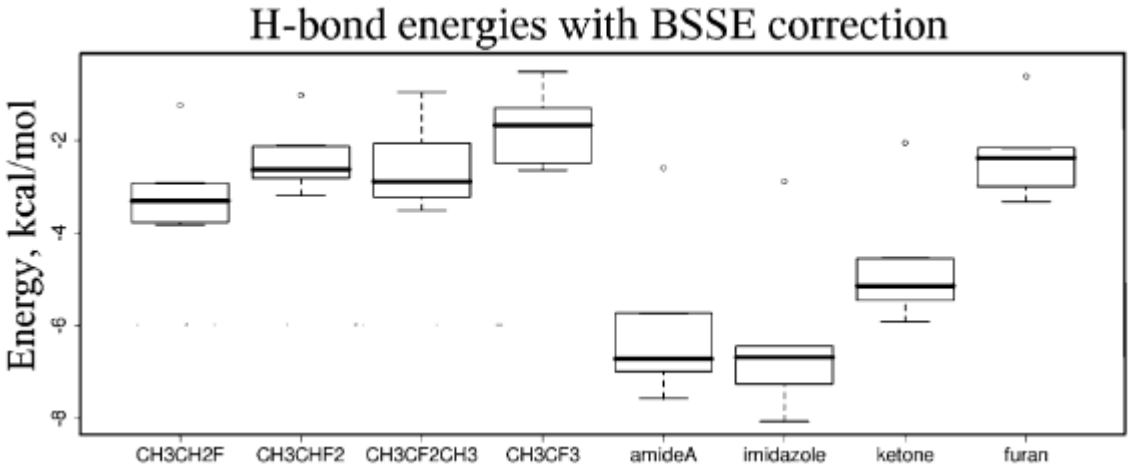
Hydrogen bond energies (kcal/mol)

Ab initio

acceptor	water	CH3CONHCH3	imidazole	EtOH	indole	CH3SH
CH3CH2F	-3.77	-2.93	-3.82	-3.34	-3.29	-1.25
CH3CHF2	-3.20	-2.12	-2.82	-2.63	-2.64	-1.03
CH3CF2CH3	-3.52	-2.06	-3.23	-2.65	-3.14	-0.96
CH3CF3	-2.65	-1.31	-1.86	-2.49	-1.50	-0.52

B3PLYP(6-311G**++)



The analysis of these data allows ranking these acceptors by hydrogen bonding properties in the following order:
imidazole > amideA > ketone > CH3CH2F > furan ~ CH3CHF2 ~ CH3CF2CH3 > CH3CF3

Samsonov, S.A. et al. J.Phys.Chem. B. 2009, 13(51):16400-8

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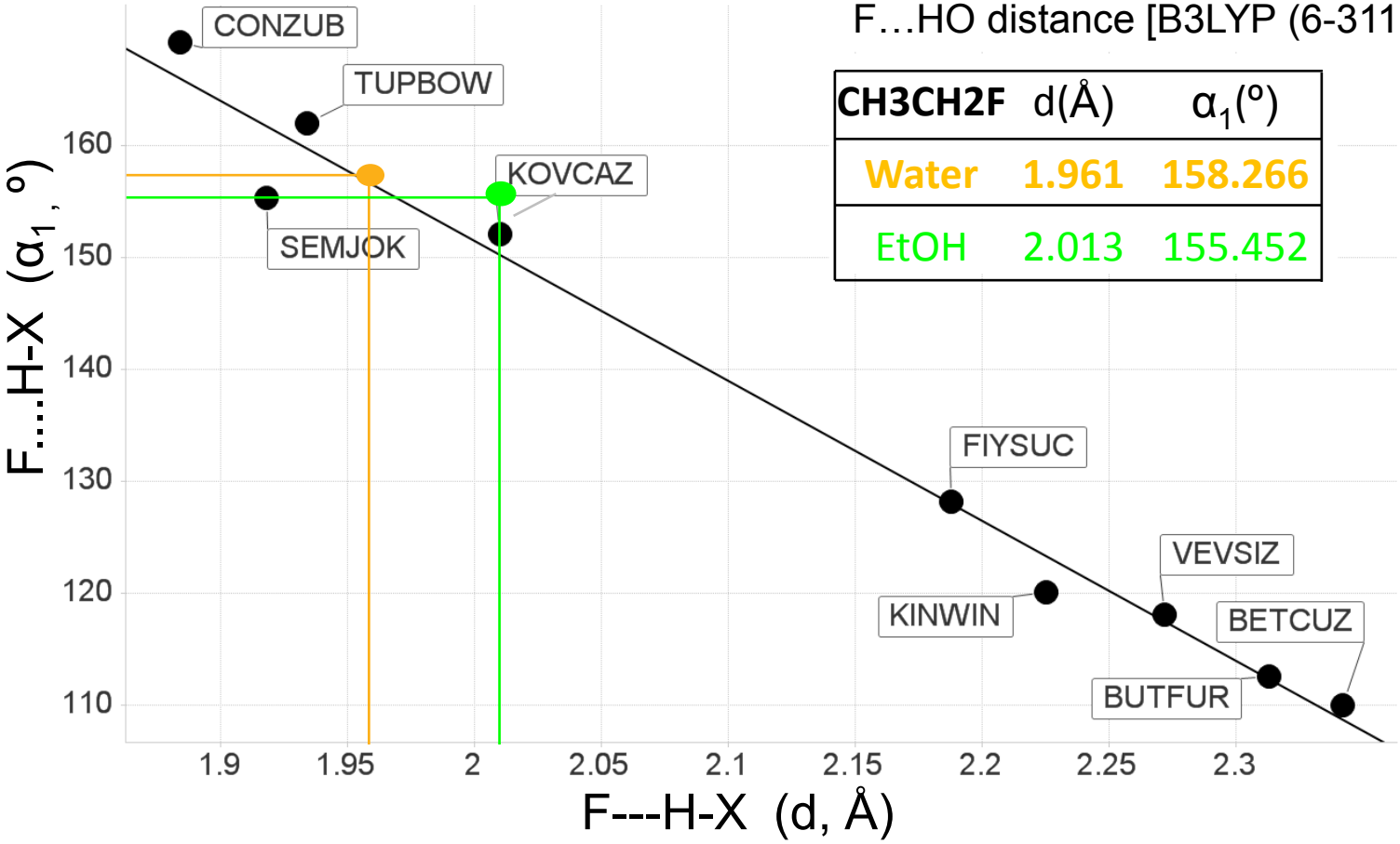
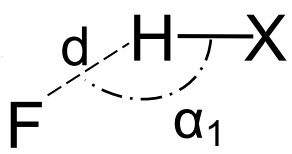
Correlation between α1 and d

Experimental vs. Ab initio



F…HO distance [B3LYP (6-311G**++), BSSE]

CH3CH2F	d(Å)	α_1(°)
Water	1.961	158.266
EtOH	2.013	155.452



Primary (R-CH_2F), shielded secondary alkyl fluorines (R'R''-CHF)

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