cresset®
software

innovative science • intuitive software

Electrostatic Complementarity™ as a fast and effective tool to optimize binding and selectivity of protein-ligand complexes

Mark Mackey

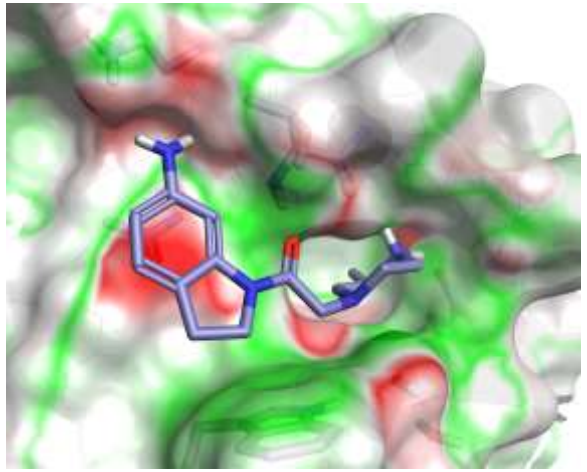
Overview

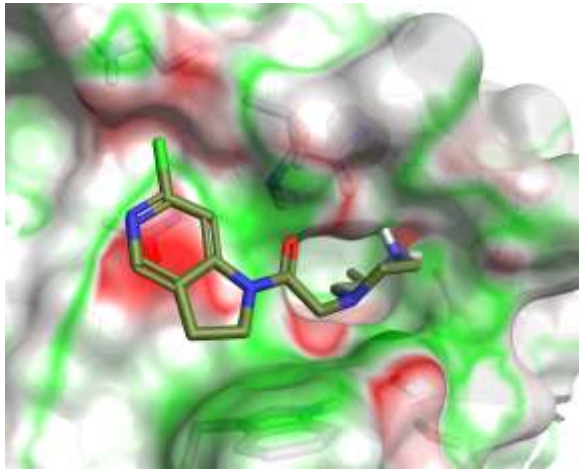
> Introduction

> Theoretical background of computing Electrostatic Complementarity™ (EC) in Flare™

> Case studies

> Conclusion and Outlook





Why electrostatic scoring?

> Electrostatic interactions between small molecules and their respective receptors are a key contributor to the binding free energy of binding ΔG (enthalpic component)

> Assessing the electrostatic match between ligands and binding pockets provides important insights into why ligands bind and what could be changed to improve binding

> Informs design of polar / enthalpic binders, which have typically better selectivity and pharmacokinetic parameters than entropic binders and were suggested to be ‘better’ drugs

Hann et al. Nat Rev Drug Discov 2012; Med Chem Comm 2011

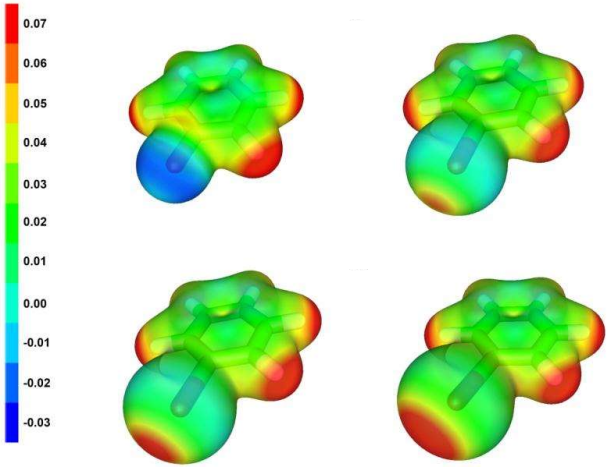
> Our clients are interested in electrostatic scoring

Electrostatic Interactions

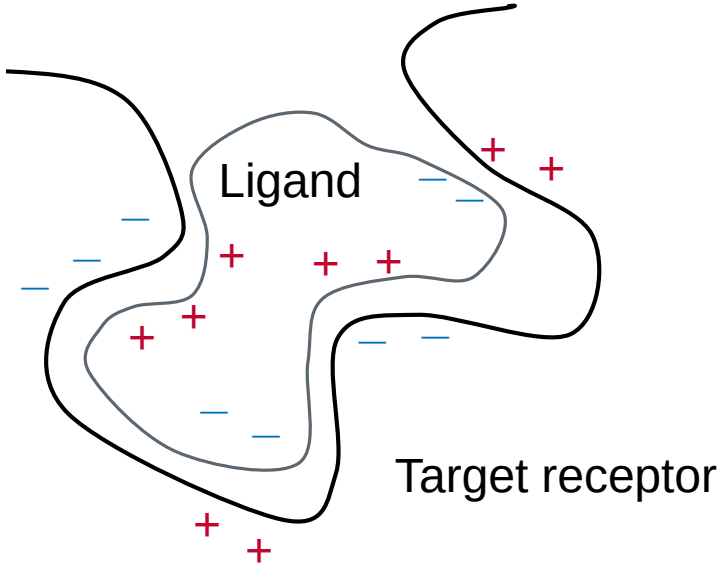
- Electrostatic interactions types
  - Hydrogen bonding (classical and weak)
  - Ionic
  - Cation-π
  - π-π
  - Lone-pair sigma-hole (e.g., halogen bonding)
  - Multipolar (e.g., fluorine bonding)

- Directionality of some electrostatic interactions can not be properly described by atom-centered charges used in classical force fields, e.g., halogen bonding, charge anisotropy of carbonyl / backbone amides, hydroxyl groups, aromatic rings

- Quantum-mechanical calculations of ESP surfaces possible but slow / expensive



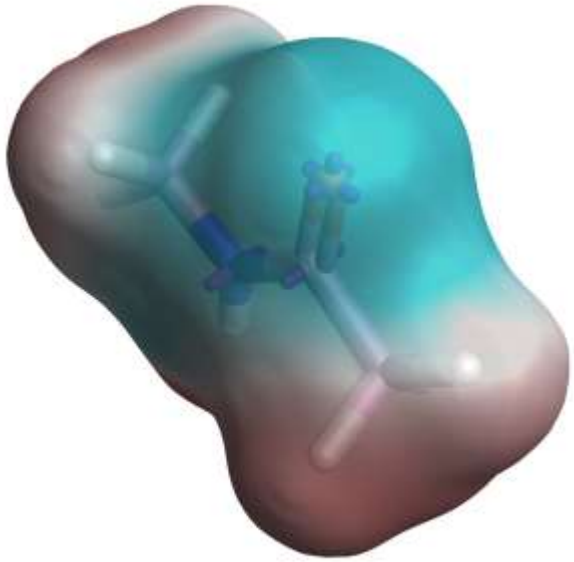
Adapted from Wilcken et al. JMC 2013

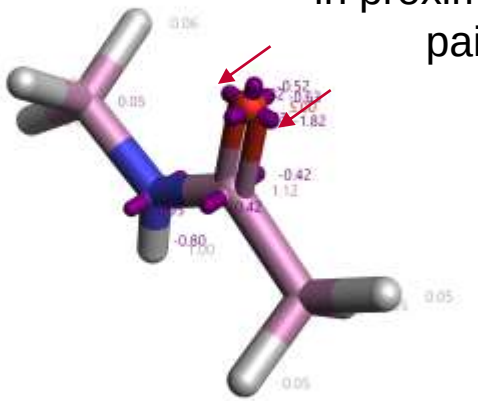


Anisotropic charge distribution with XED force field

> The polarizable XED force-field is an excellent base for calculating electrostatic properties

  > Description of anisotropic atomic charge distributions at relatively modest computational costs



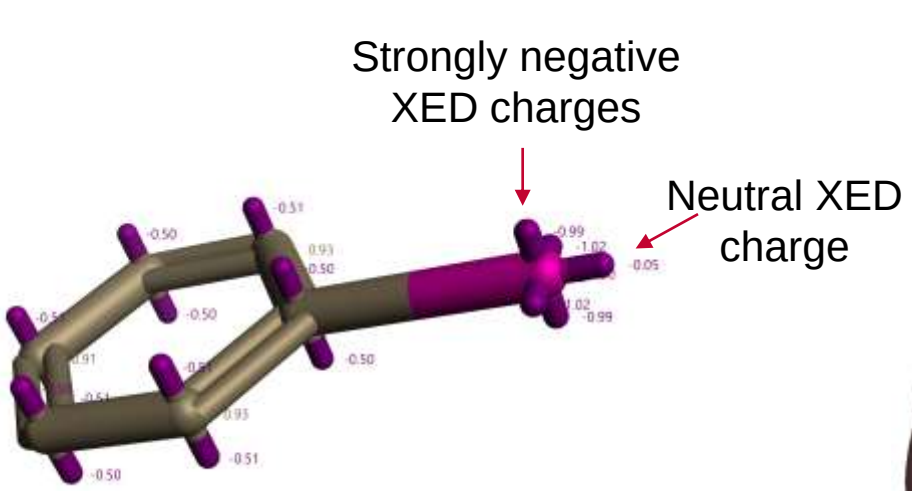


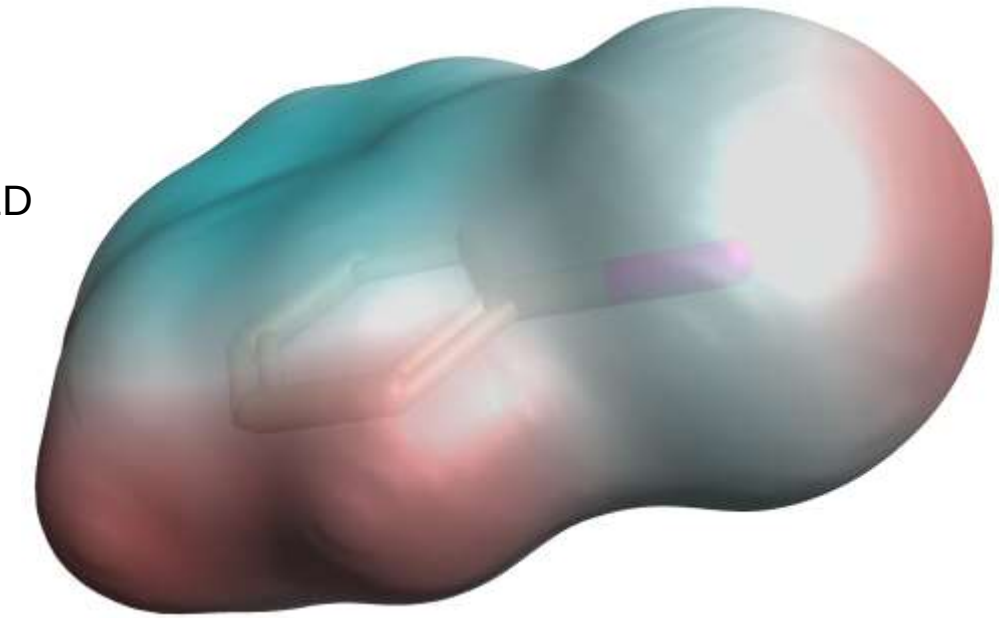
XED charges more negative in proximity to oxygen lone pair orientation

Anisotropic charge distribution with XED force field

> The polarizable XED force-field is an excellent base for calculating electrostatic properties

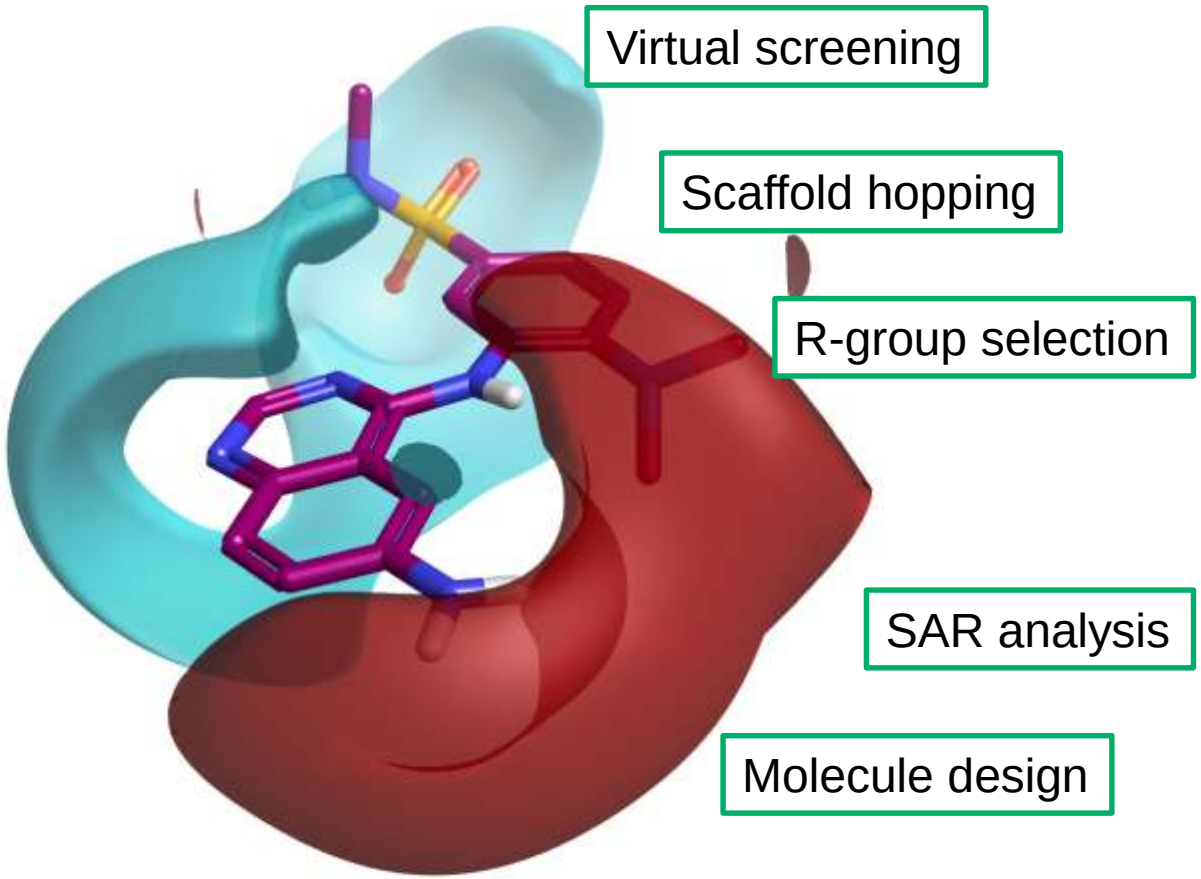
  > Description of anisotropic atomic charge distributions at relatively modest computational costs



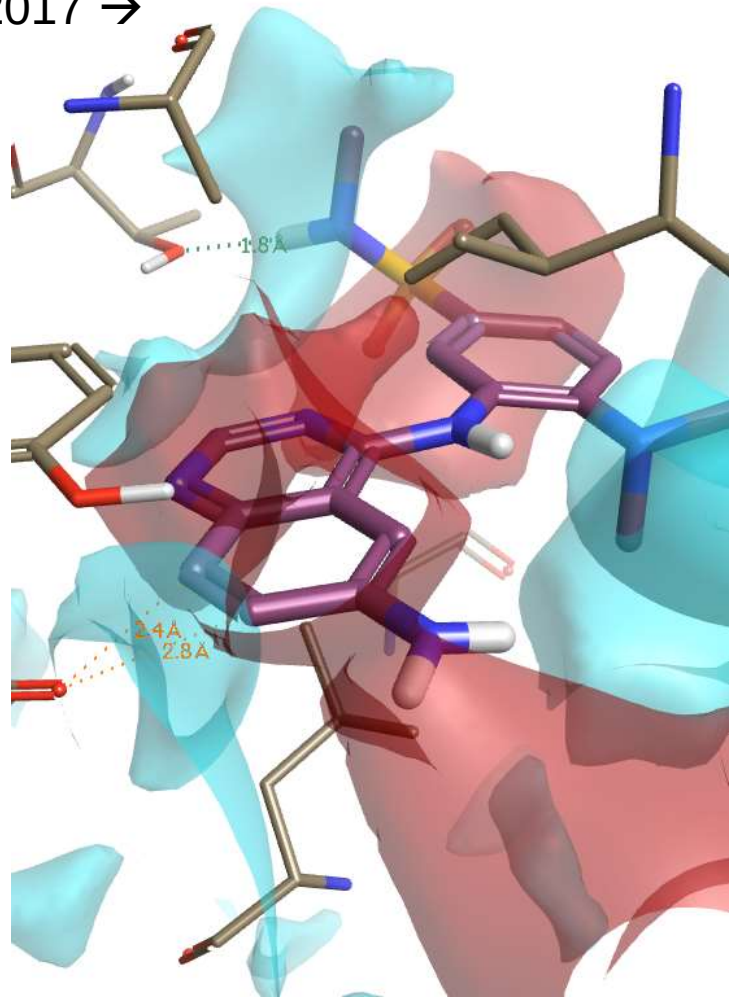


Ligand and protein molecular interaction potentials

2006 →



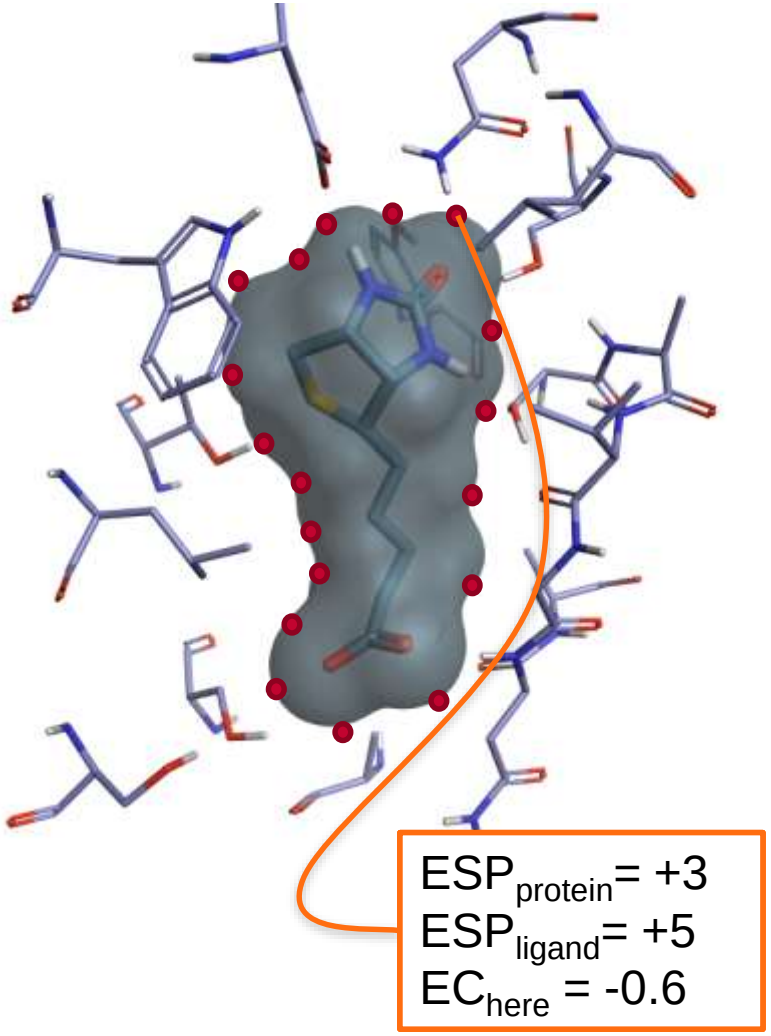
2017 →



Positive potential Negative potential

Calculating Electrostatic Complementarity

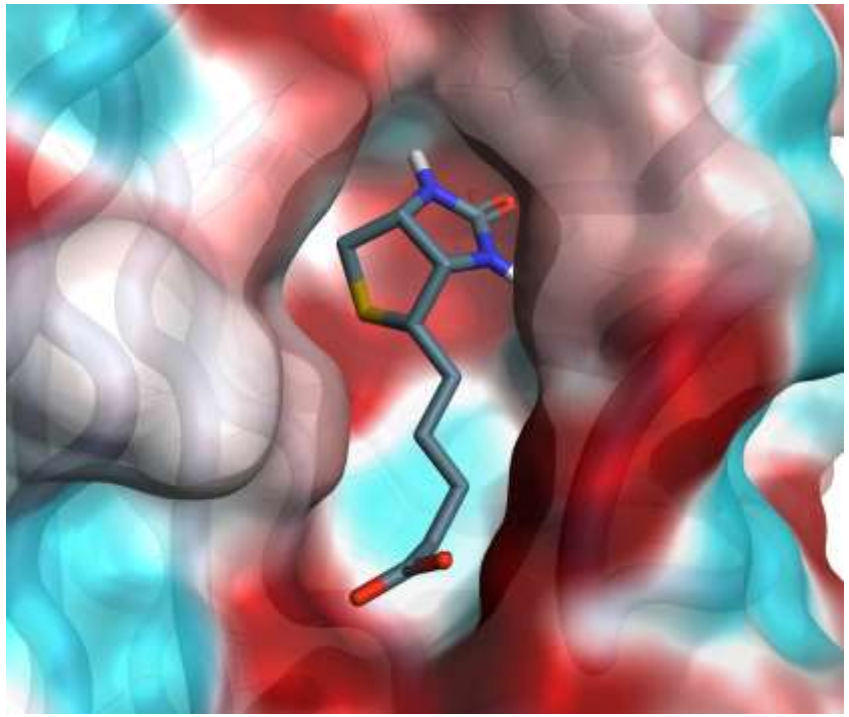
1. Place a solvent-accessible surface on the ligand
2. For each vertex on the surface, compute the electrostatic potential due to the ligand and to the protein
3. Scale down points on the ligand surface which are too far away from any protein atom (≥ 3 Å)
4. Cap values to a maximum (roughly corresponding to the maximum potential of a water molecule)
5. Complementarity(vertex) = $\left(1 - \frac{ESP_{ligand} + ESP_{protein}}{MAX\,(ESP_{ligand,protein})}\right)$
6. Color vertices according to complementarity
   - → *perfect electrostatic complementarity = 1 (green)*
   - → *both potentials zero = 0 (white)*
   - → *perfect electrostatic clash = -1 (red)*



Biotin-Streptavidin example

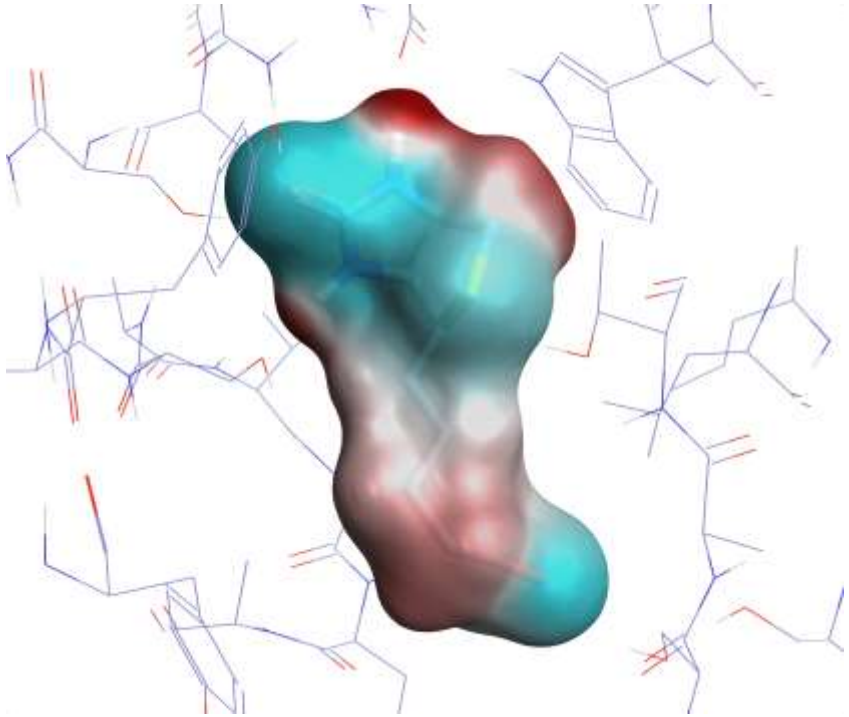
> Visual inspection of electrostatic potential (Biotin-Strepavidin)

→ red = positive potential and blue = negative potential



XED ESP surface of Streptavidin

180˚ flip

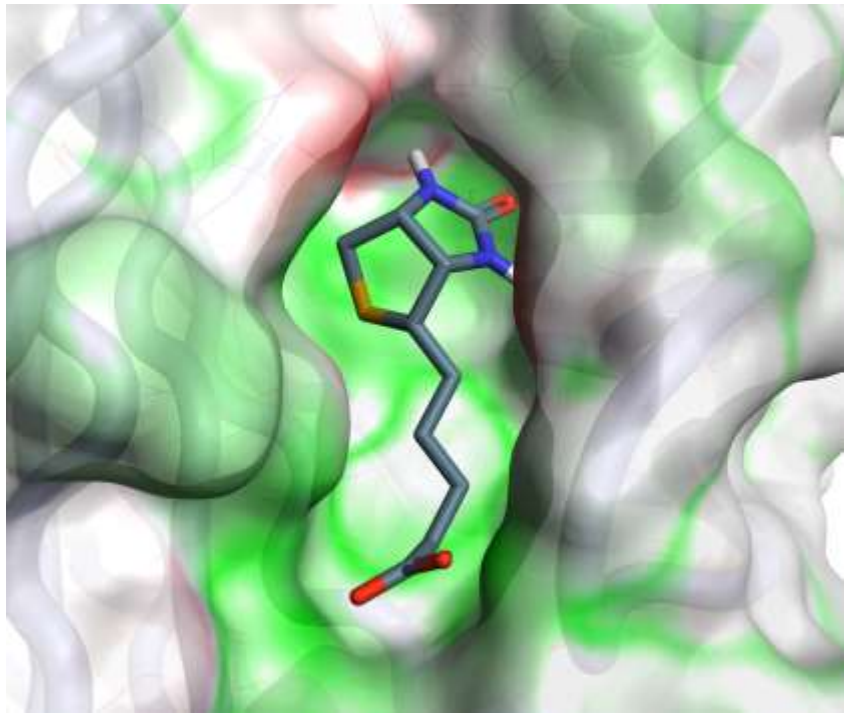


XED ESP surface of Biotin

Biotin-Streptavidin example

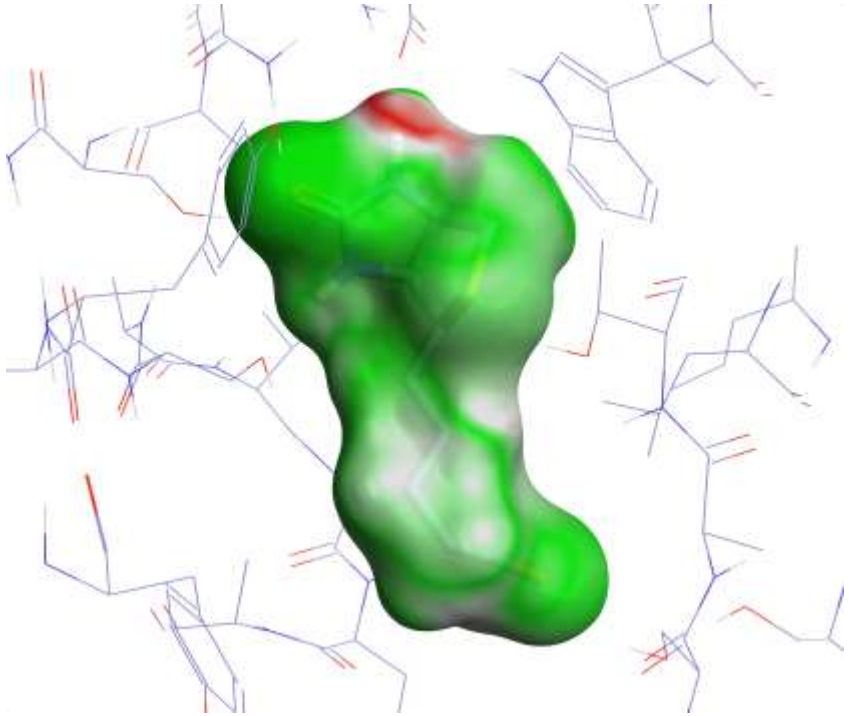
> Visualization of electrostatic complementarity (Biotin-Strepavidin)

→ green = good complementarity and red = bad complementarity



EC surface of Streptavidin

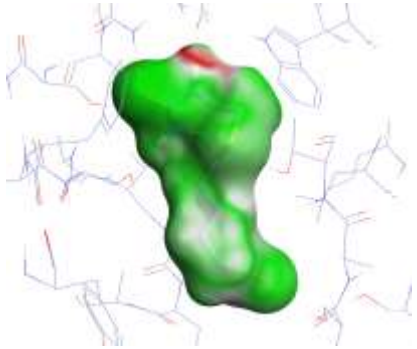
180˚ flip



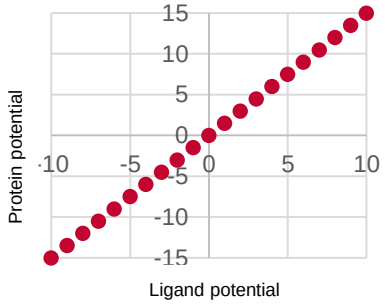
EC surface of Biotin

Converting Electrostatic Complementarity colors to scores

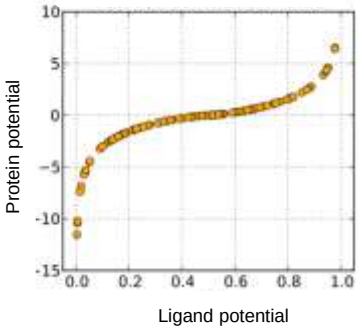
- Complementarity score (-1,1)
  - Normalized surface integral of the complementarity score described before
  - Includes some compensation for desolvation effects (capping of electrostatic potential values), and so may be more robust when these are significant
- Complementarity r (-1,1) or Pearson
  - Pearson correlation coefficient of protein and ligand electrostatic potentials sampled on the surface vertices
  - Can provide a better indication of ligand activity in some cases but is susceptible to noise
- Complementarity rho (-1,1) or Spearman
  - Spearman rank correlation coefficient of protein and ligand electrostatic potentials sampled on the surface vertices
  - More robust against background electric fields (useful if the computed protein electric potential is being biased by a large net charge on the protein)



Pearson



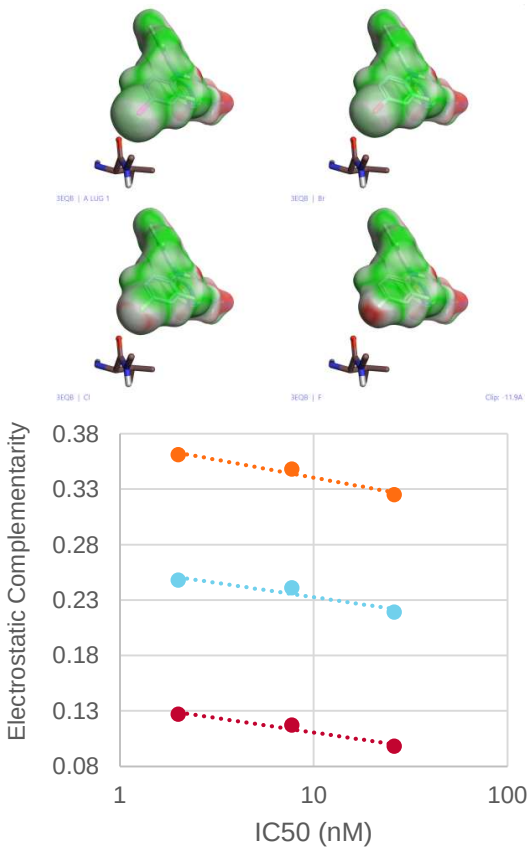
Spearman



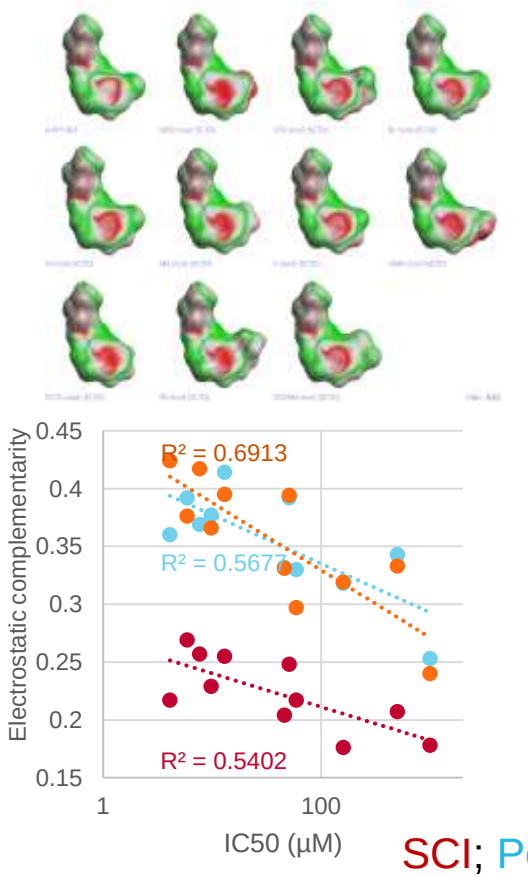
Case studies

> Application to several data sets

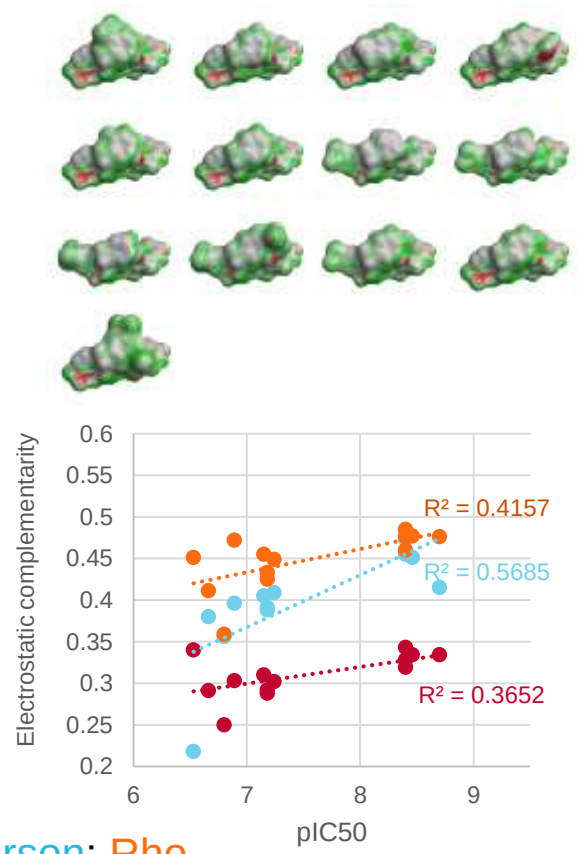
Halogen Bonding



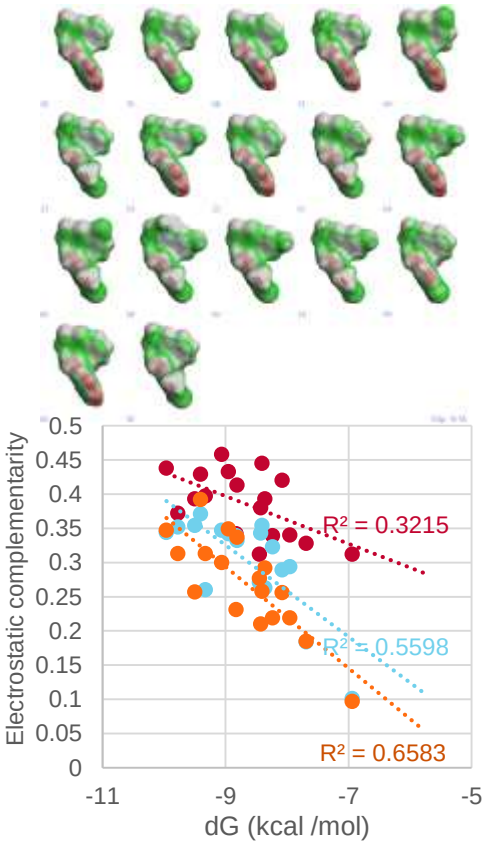
XIAP



DPP4



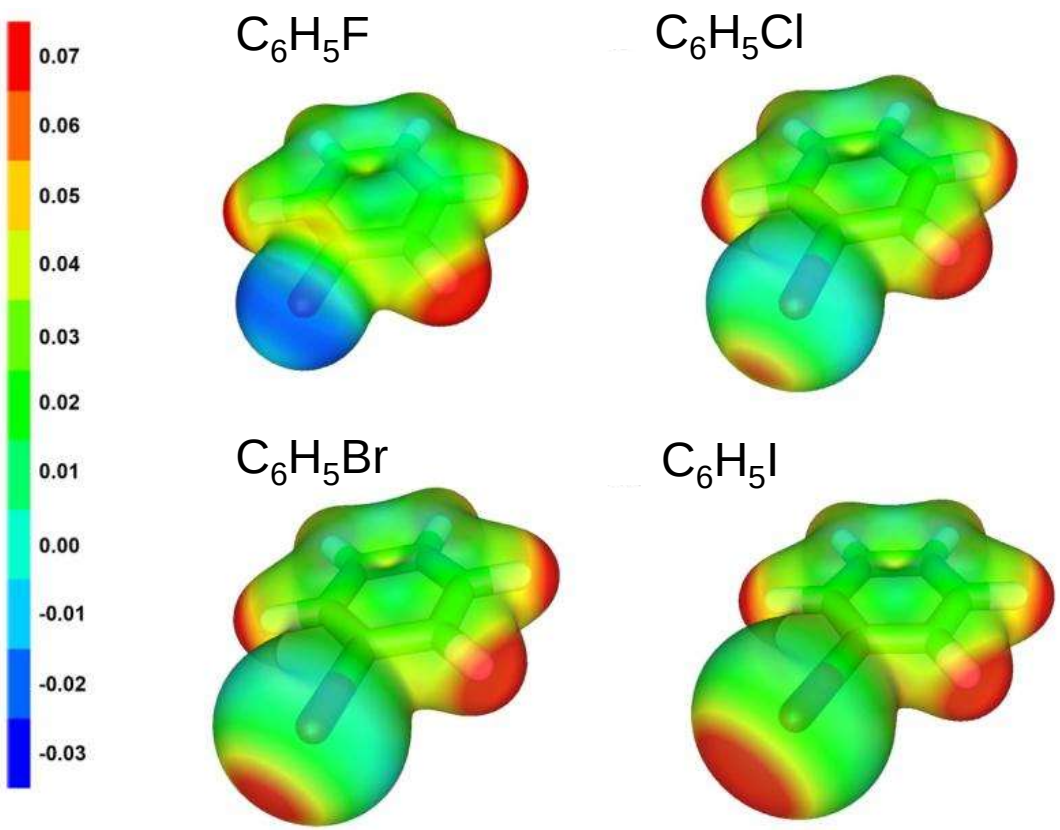
MCL1

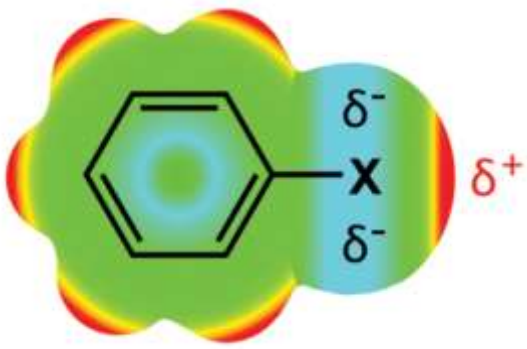


SCI; Pearson; Rho

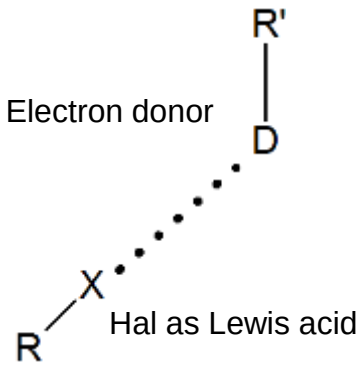
Halogen Bonding example

> Concept





Driven by the σ-hole (anisotropy of electron density on halogen)



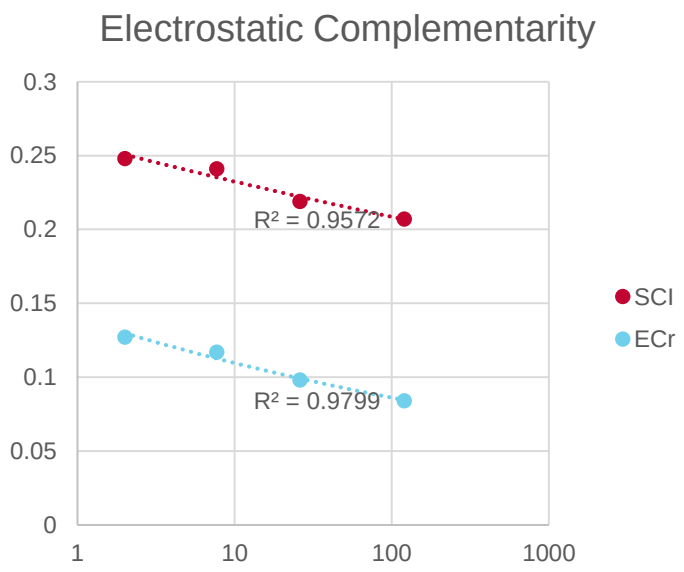
> Interesting case for electrostatic interactions
  > Sigma hole increases from Cl to I
  > Halogen bond strength increases from Cl to I

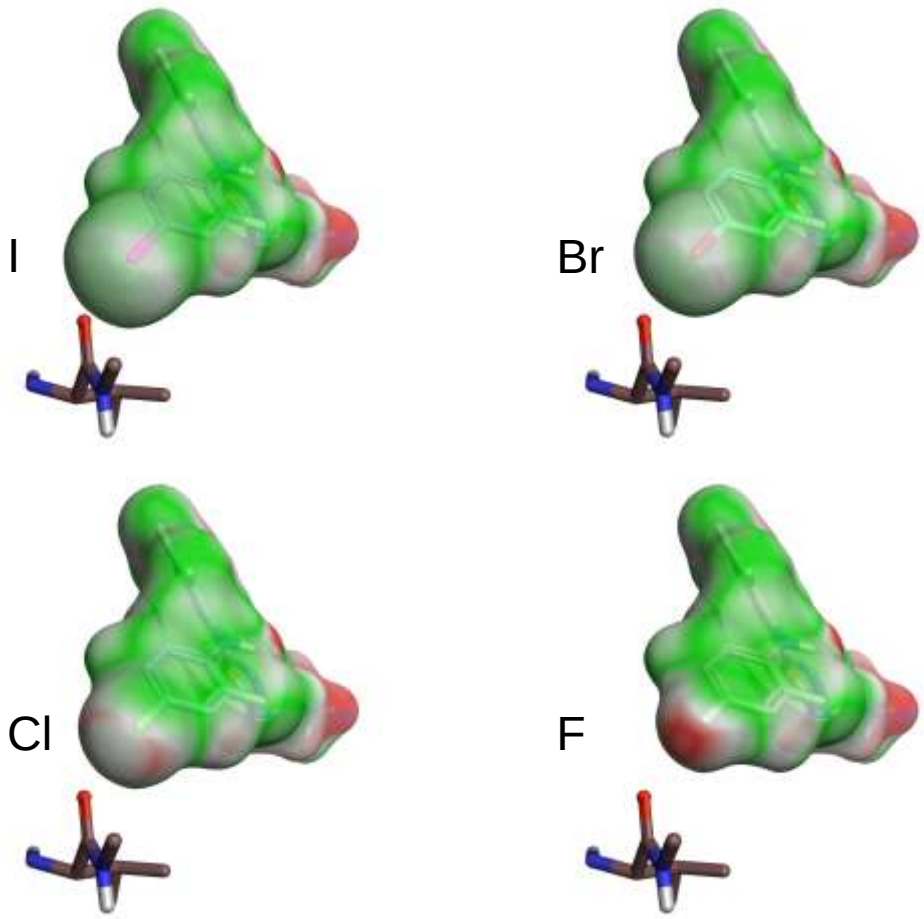
Wilcken et al. JMC 2013

Halogen Bonding example

MEK1

Receptor	Substituent	IC50	SCI	ECr
3EQB	F	120	0.207	0.084
3EQB	Cl	26	0.219	0.098
3EQB	Br	7.7	0.241	0.117
3EQB	I	2	0.248	0.127

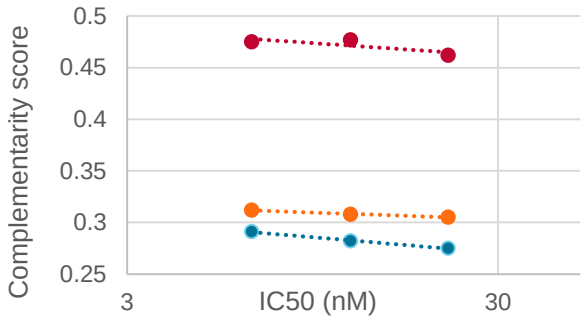


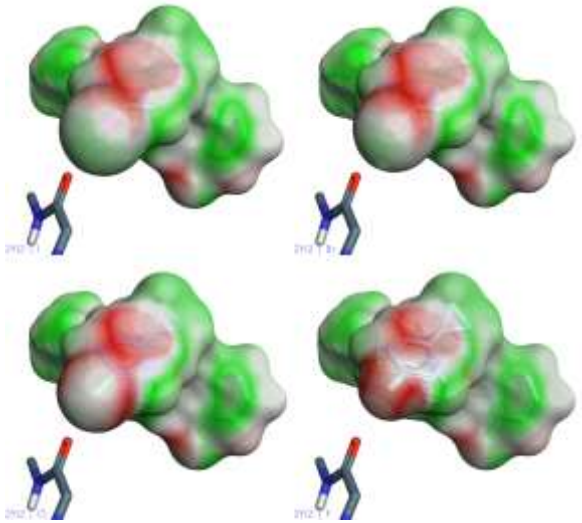


Hardegger et al. ChemMedChem 2011

Halogen Bonding example

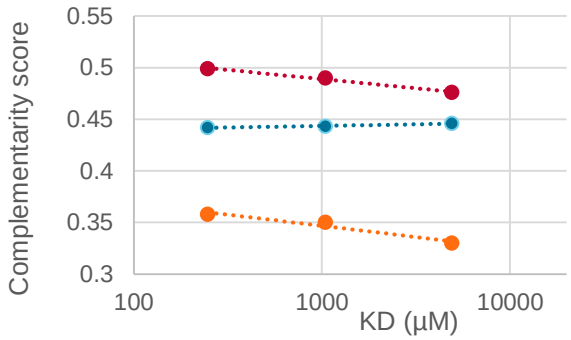
> Cathepsin-L example

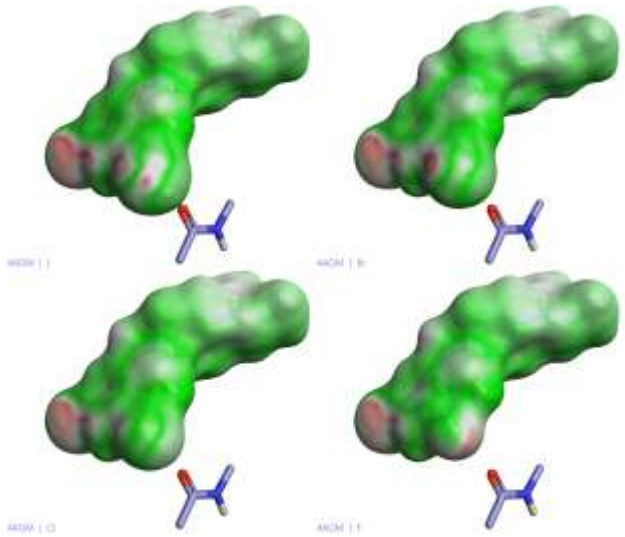




Hardegger *et al*. ChemMedChem 2011

> p53-Y220C example

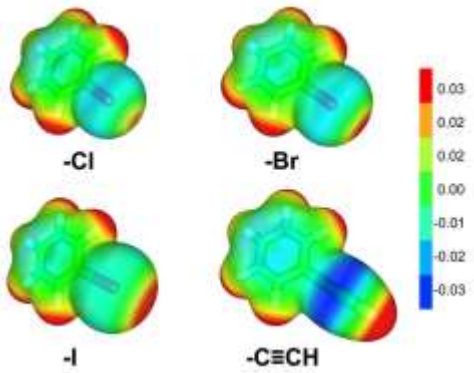




Wilcken *et al.* JMC Perspective 2013

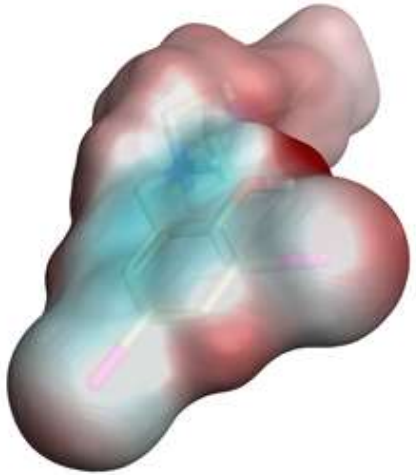
Halogen bioisosterism

> p53-Y220C: Ethynyl as halogen bioisostere

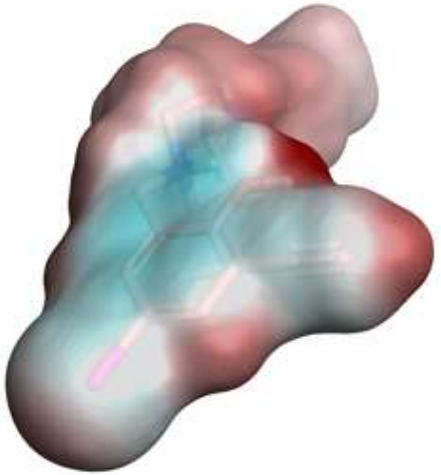


QM ESP surface

XED ESP surface

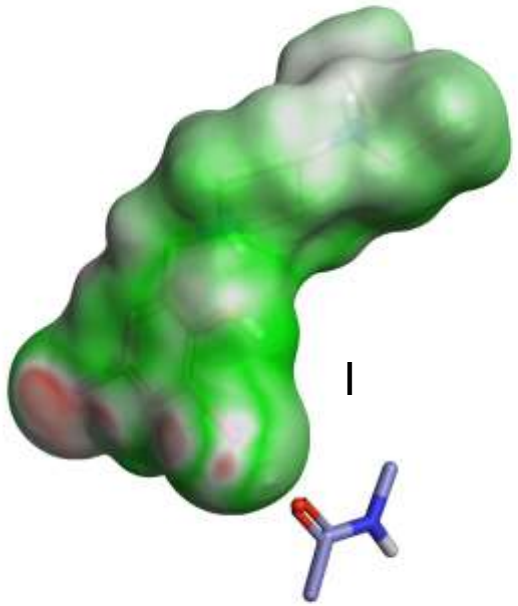


I

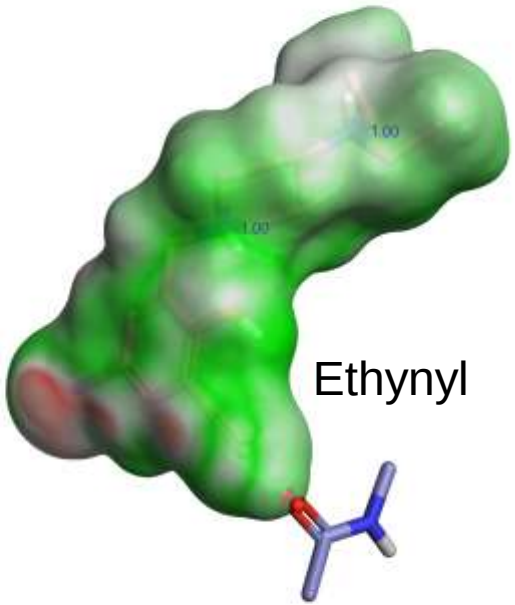


Ethynyl

Similar electrostatic complementarity



I



Ethynyl

Wilcken et al. ACS Chem Biol 2015

Application to a series of XIAP inhibitors

Journal of Medicinal Chemistry

Article

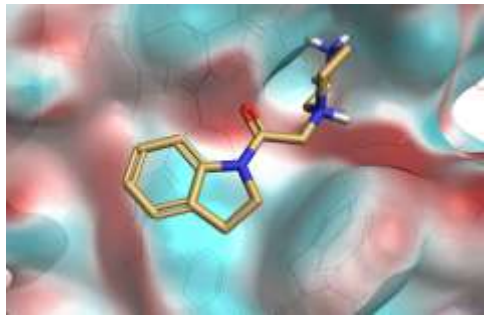
pubs.acs.org/jmc

Fragment-Based Drug Discovery Targeting Inhibitor of Apoptosis Proteins: Discovery of a Non-Alanine Lead Series with Dual Activity Against cIAP1 and XIAP

Gianni Chessari,* Ildiko M. Buck, James E. H. Day, Philip J. Day, Aman Iqbal, Christopher N. Johnson, Edward J. Lewis, Vanessa Martins, Darcey Miller, Michael Reader, David C. Rees, Sharna J. Rich, Emiliano Tamanini, Marc Vitorino, George A. Ward, Pamela A. Williams, Glyn Williams, Nicola E. Wilsher, and Alison J.-A. Woolford

Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, United Kingdom

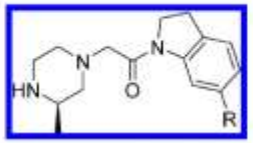
> Investigation of the electronegative pocket of XIAP-BIR3 by modulating the functionality of the indole C6 with a range of electron withdrawing and electron donating substituents.



Negative potential

Positive potential

Table 2. XIAP-BIR3 Affinity of Substituted Indolines 7−16

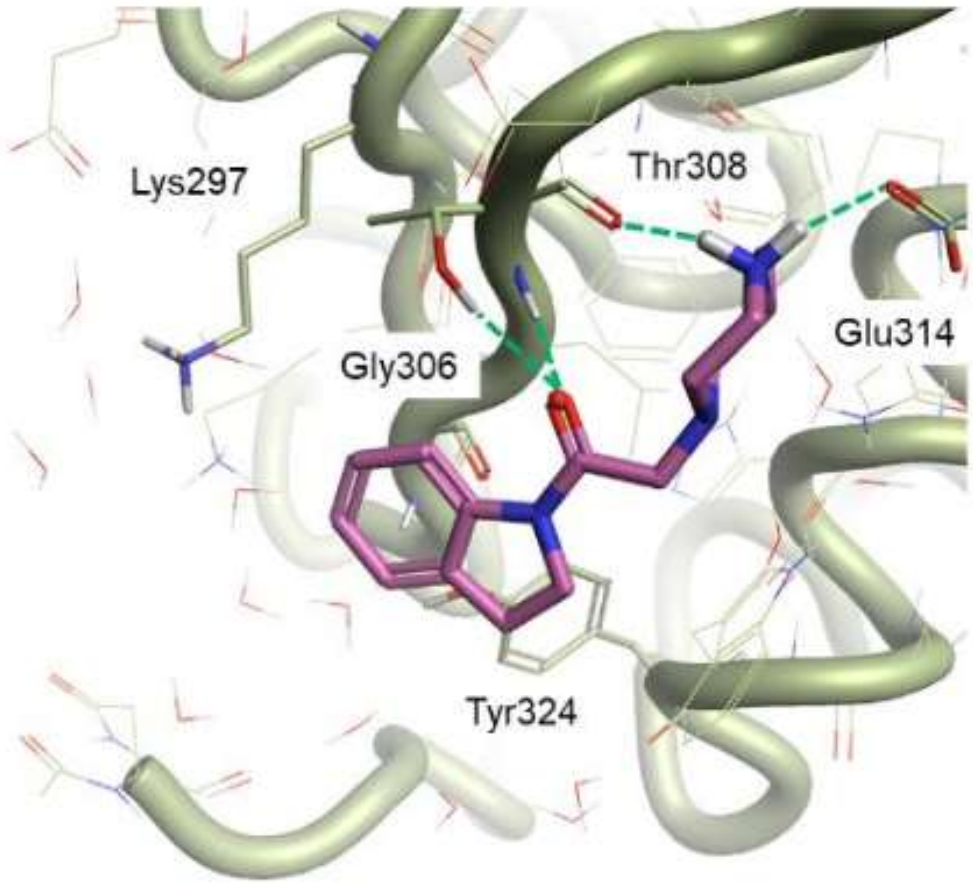


compd	R	Hammett σ_p	XIAP-BIR3[a] IC_{50} (μM) or %I	XIAP-BIR3 LE[b] (kcal mol^{-1} per non-H atom)
7	−H	0.00	52% @ 495 μM	~0.24
8	$-NH_2$	−0.66	56% @ 1000 μM	~0.20
9	−OMe	−0.27	49% @ 155 μM	~0.25
10	−Me	−0.17	46	0.30
11	−iPr	−0.15	59	0.26
12	−F	0.06	51	0.29
13	−Cl	0.23	13	0.33
14	−Br	0.23	9.8	0.34
15	$-CF_3$	0.54	5.9	0.31
16	$-SO_2Me$	0.72	4.1	0.32

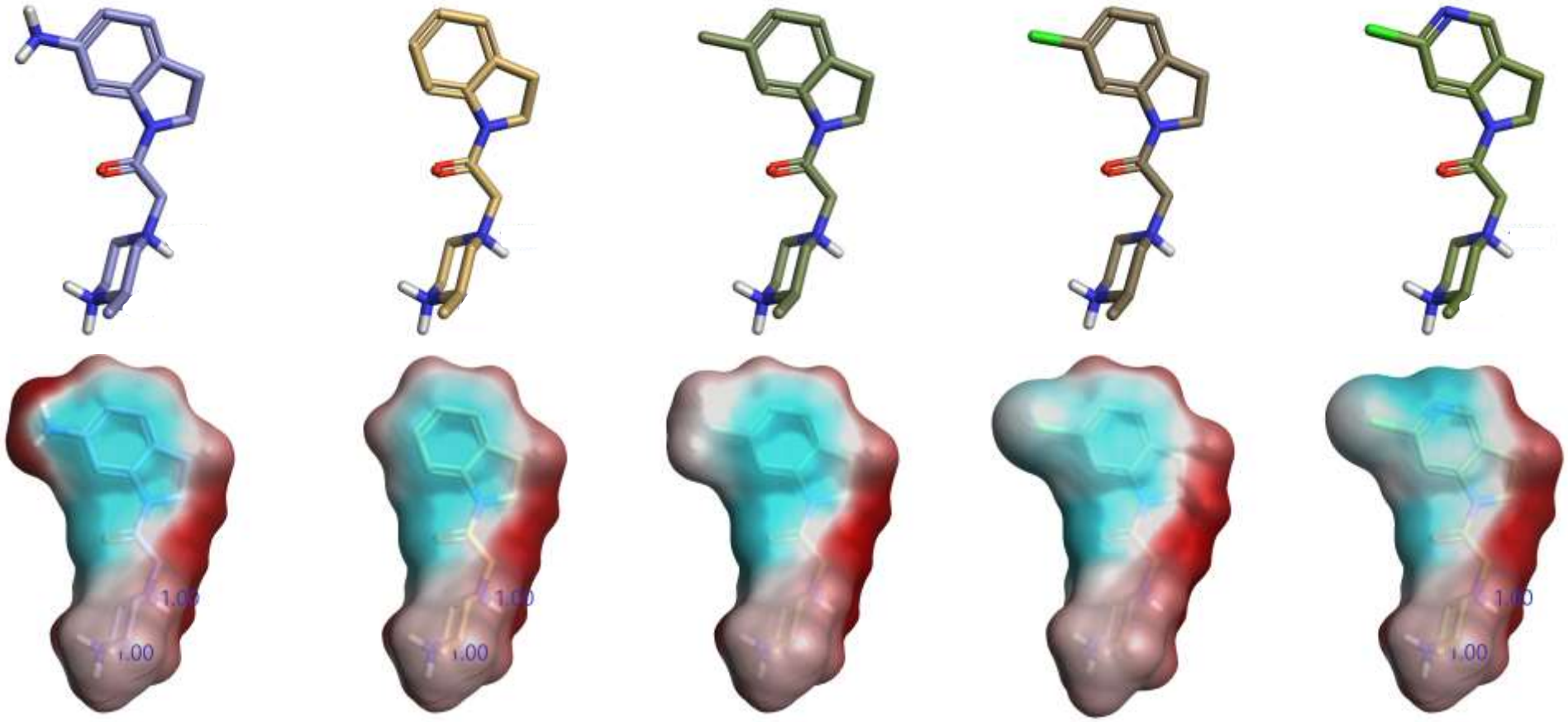
[a]Values were determined by fluorescence polarization assay (see Experimental Section). Potency data are reported as the mean of at least two runs.
[b]Values calculated according to the Hopkins formula.[17]

Data set and experimental set-up

- Table 2 compounds compared to 5C7A protein
- The side chain atoms were minimized with the XED force field for each ligand as many modelled binding modes clash with the flexible side chain of Lys297.
- Retained water have at least 2 H-bond contacts to the protein or at least 1 H-Bond to ligand and protein.
- Manual building of ligands
  - Substructure alignment of the indoline scaffold to the 5C7A ligand using Forge



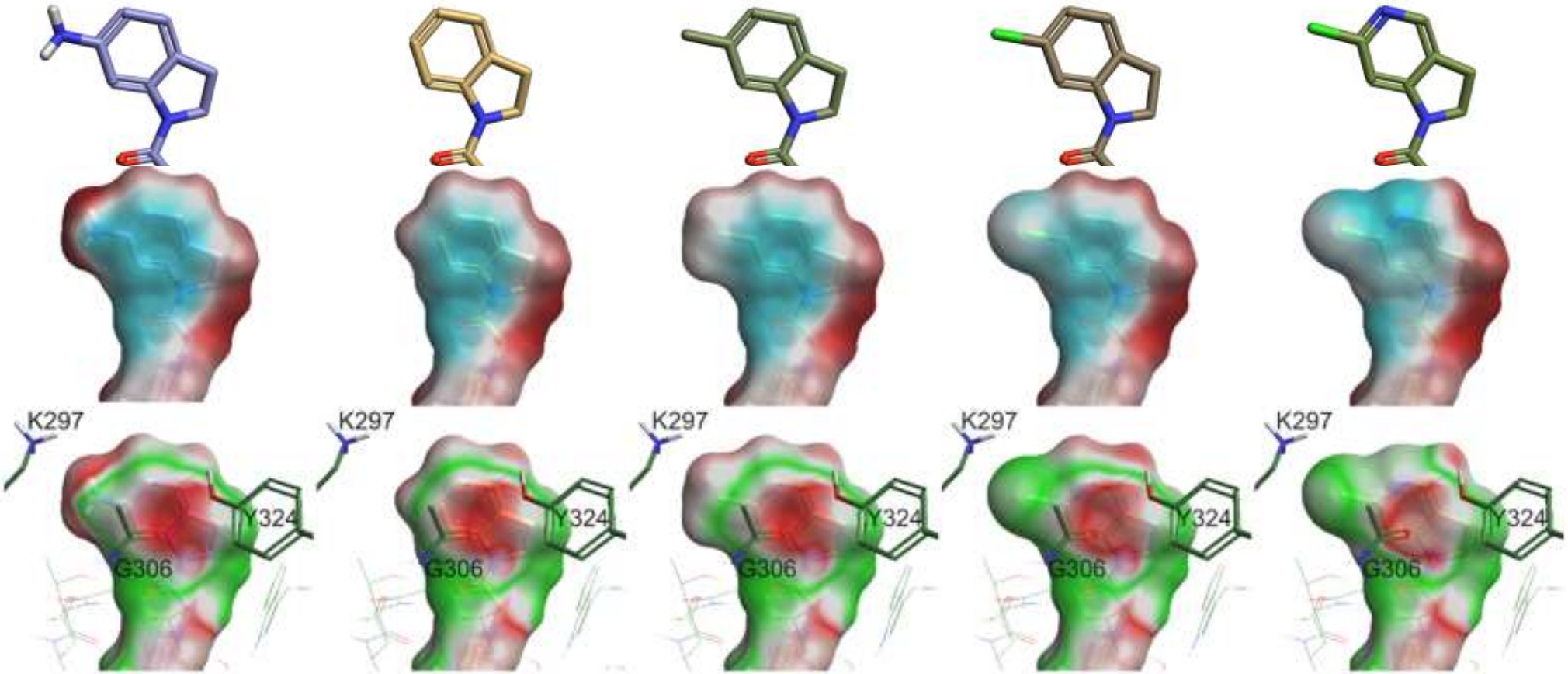
Electrostatic potential of five XIAP inhibitors



Compound 8	Compound 7	Compound 10	Compound 13	Compound 17
pIC_{50} = 3.0	3.3	4.3	4.5	5.1

→ Increase electron withdrawing effect

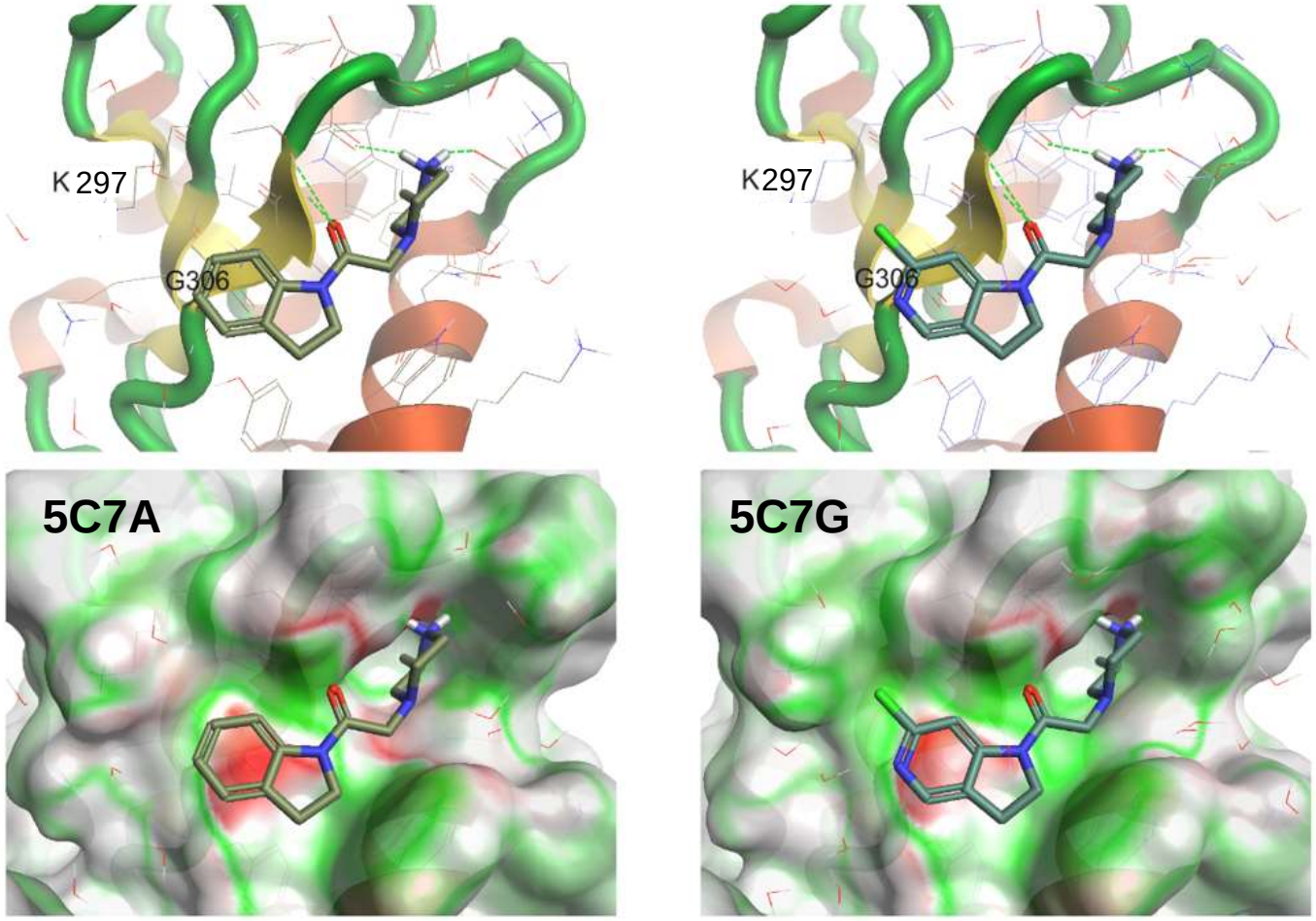
Electrostatic Complementarity of five XIAP inhibitors



Compound 8	Compound 7	Compound 10	Compound 13	Compound 17
pIC_{50} = 3.0	pIC_{50} = 3.3	pIC_{50} = 4.3	pIC_{50} = 4.9	pIC_{50} = 5.1
SCI = 0.17	SCI = 0.2	SCI = 0.2	SCI = 0.24	SCI = 0.25
R = 0.25	R = 0.33	R = 0.32	R = 0.38	R = 0.36
Rho = 0.23	Rho = 0.32	Rho = 0.32	Rho = 0.37	Rho = 0.41

EC to XIAP binding site

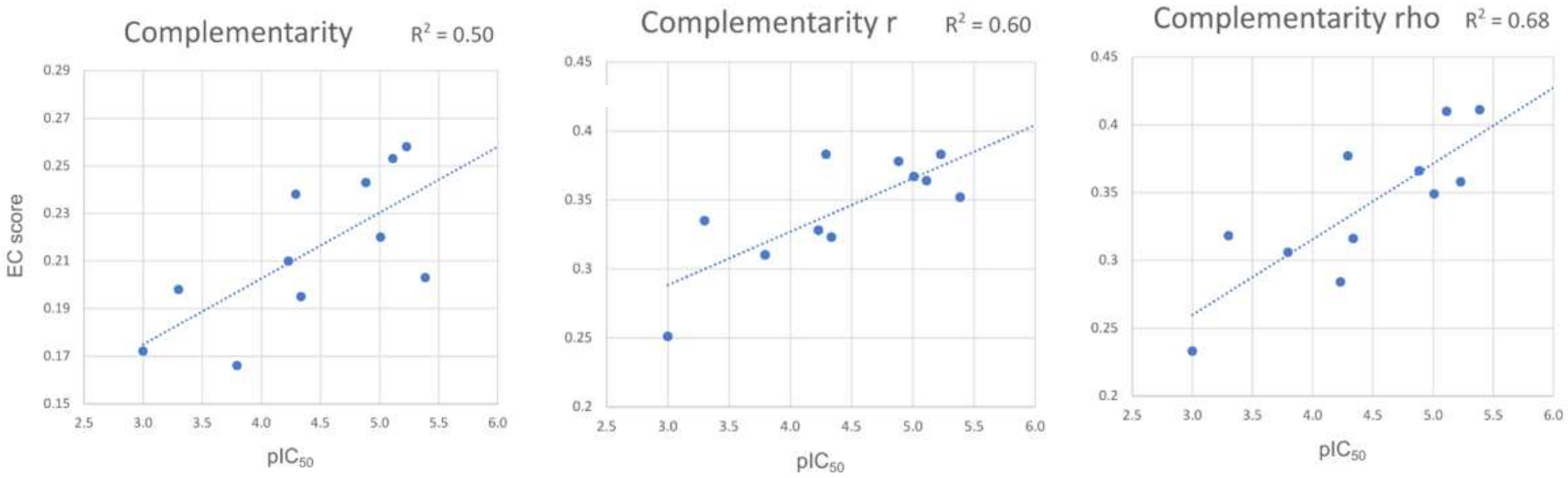
- The EC maps show improved EC
  - Around Lys297 side chain
  - Around Gly306 backbone



Compound 7 pIC_{50}= 3.3
LE = 0.24

Compound 17 pIC_{50} = 5.1
LE = 0.35

EC scores and pIC_{50} correlation for the XIAP series



> Nice correlation between the XIAP-BIR3 pIC_{50} and the EC scores
> EC maps provide a visual insight into ligand - protein binding and activity prediction
> Calculations of EC scores are fast - just over 1 second per molecule

Application to a series of mGLU5 negative allosteric modulators

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

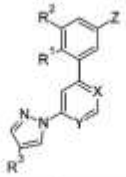
Fragment and Structure-Based Drug Discovery for a Class C GPCR: Discovery of the $mGlu_5$ Negative Allosteric Modulator HTL14242 (3-Chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile)

John A. Christopher,* Sarah J. Aves, Kirstie A. Bennett, Andrew S. Doré, James C. Errey, Ali Jazayeri, Fiona H. Marshall, Krzysztof Okrasa, Maria J. Serrano-Vega, Benjamin G. Tehan, Giselle R. Wiggin, and Miles Congreve

Heptares Therapeutics Ltd., BioPark, Welwyn Garden City, Hertfordshire AL7 3AX, U.K.

> Two ligand-bound X-ray structures with 2.6 and 3.1 A resolution (clear density for ligands)

Table 1. In Vitro Profile of Compounds 6−17

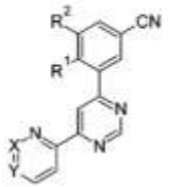


PDB: 5CGC

	X	Y	Z	R^1	R^2	R^3	$mGlu_5$ pK_i	$mGlu_5$ pIC_{50}	RLM $t_{1/2}$ (min)
6	N	N	CN	H	F	H	7.2	6.4	25
7	N	N	CN	F	H	H	6.6	nd[a]	6
8	N	N	CN	H	H	H	6.1	nd	nd
9	N	N	H	H	F	H	5.1	nd	nd
10	N	N	OMe	H	F	H	<4.2	nd	nd
11	N	N	$CONH_2$	H	F	H	<4.2	nd	nd
12	N	N	CN	H	Me	H	8.4	7.9	10
13	N	N	CN	H	Cl	H	8.4	8.3	12
14	N	N	CN	F	Cl	H	9.3	8.6	20
15	N	CH	CN	H	Me	H	7.6	7.4	51
16	CH	N	CN	H	Me	H	7.7	7.7	43
17	N	N	CN	H	Cl	F	7.7	7.1	52

[a]nd = not determined.

Table 2. In Vitro Profile of Compounds 21−30



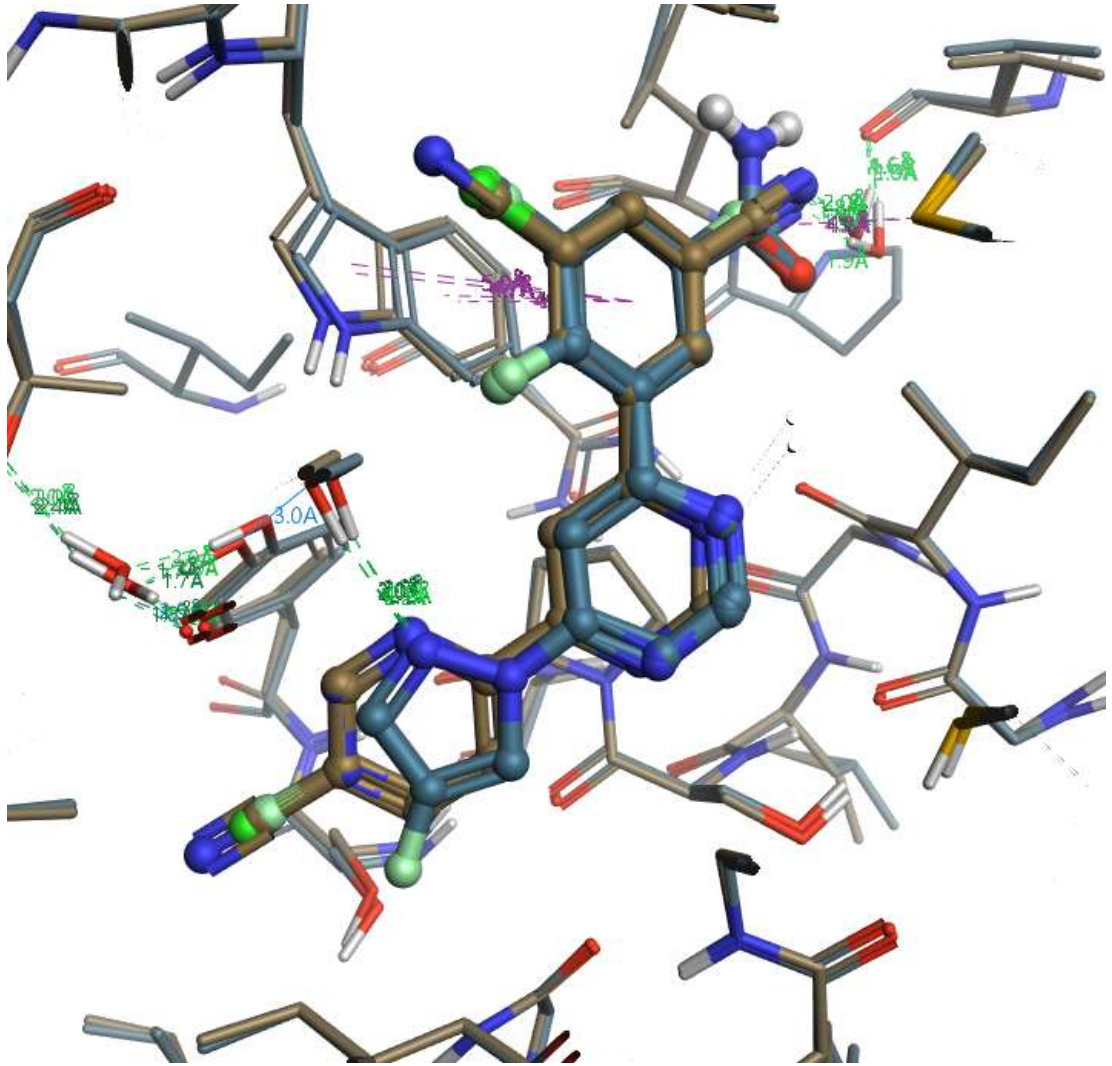
PDB: 5CGD

	X	Y	R^1	R^2	$mGlu_5$ pK_i	$mGlu_5$ pIC_{50}	RLM $t_{1/2}$ (min)
21	CH	CH	H	Cl	8.5	8.6	43
22	CH	CH	F	Cl	8.9	8.8	31
23	CH	CMe	H	Cl	8.6	8.3	19
24	CH	CF	H	CN	8.8	8.6	>100
25	CH	CF	H	Cl	9.3	9.2	44
26	CH	CCN	H	Cl	9.2	9.2	>100
27	CH	CCl	H	Cl	8.8	8.5	35
28	CH	CF	F	Cl	9.1	9.4	87
29	N	CH	H	Cl	8.0	6.7	nd[a]
30	CH	N	H	Cl	8.3	7.5	nd

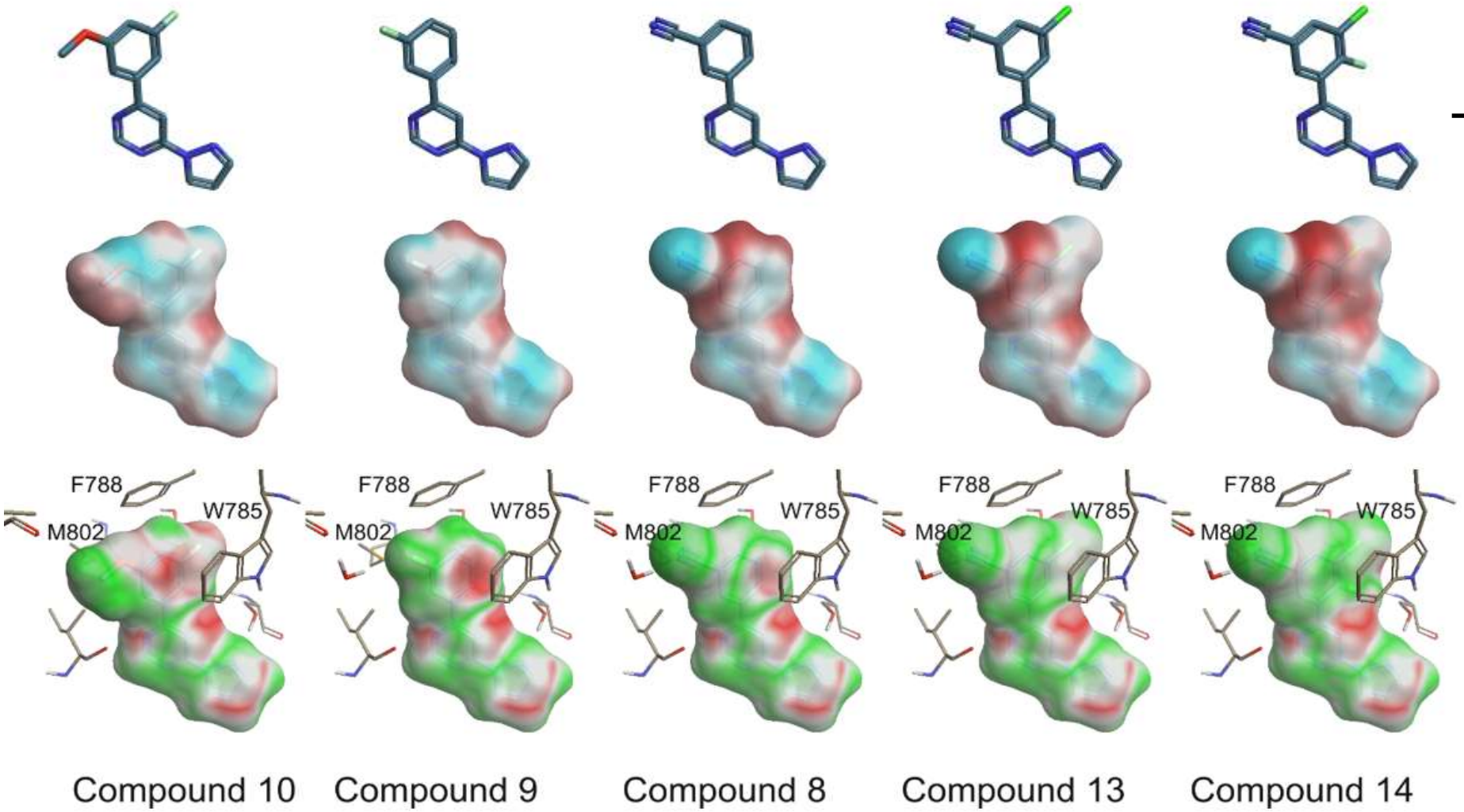
[a]nd = not determined.

Data set and experimental set-up

- Table 1 compounds compared to 5CGC protein
- Table 2 compounds compared to 5CGD protein
- Only minor changes in structure
- Retained “stable” water from 3D RISM calculation (same waters in each structure)
- Manual mutation of ligands
  - No optimization of binding
  - Manual orientation of groups



Electrostatic Complementarity of five mGLU5 NAMs

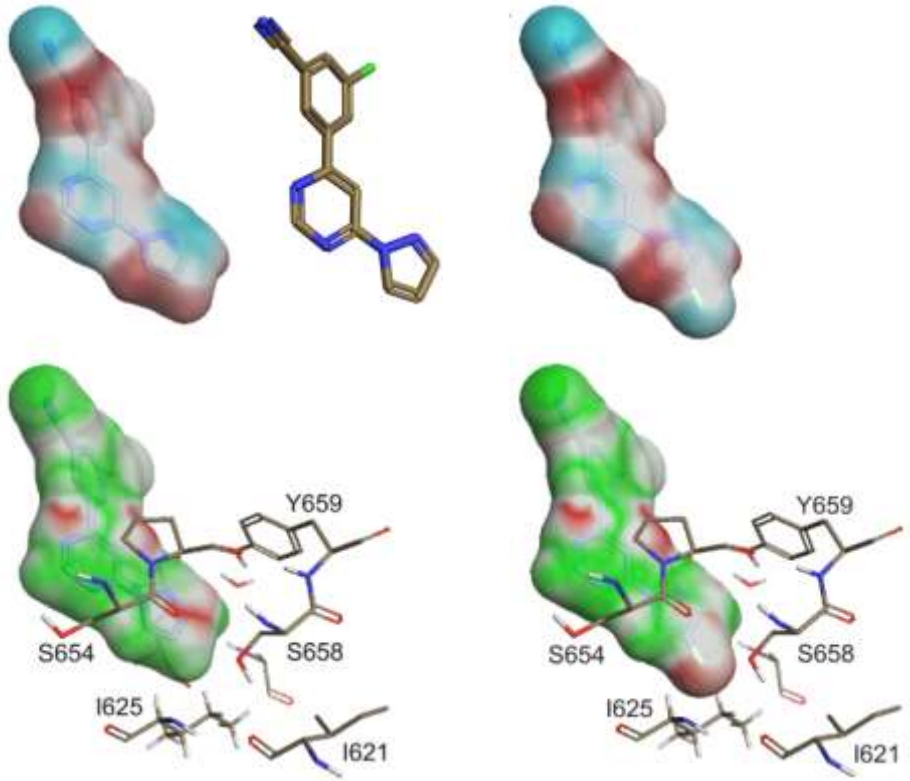


Increasing electron withdrawing effect

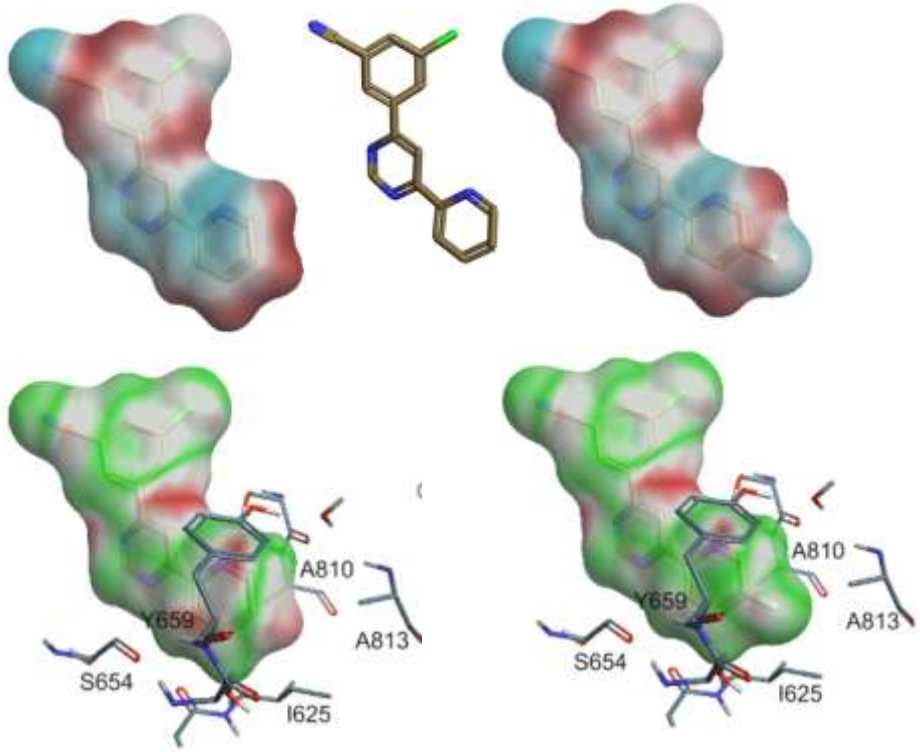
Compound 10	Compound 9	Compound 8	Compound 13	Compound 14
pKi <4.2	5.1	6.1	8.4	9.3

Impact of fluorination on EC and activities

A

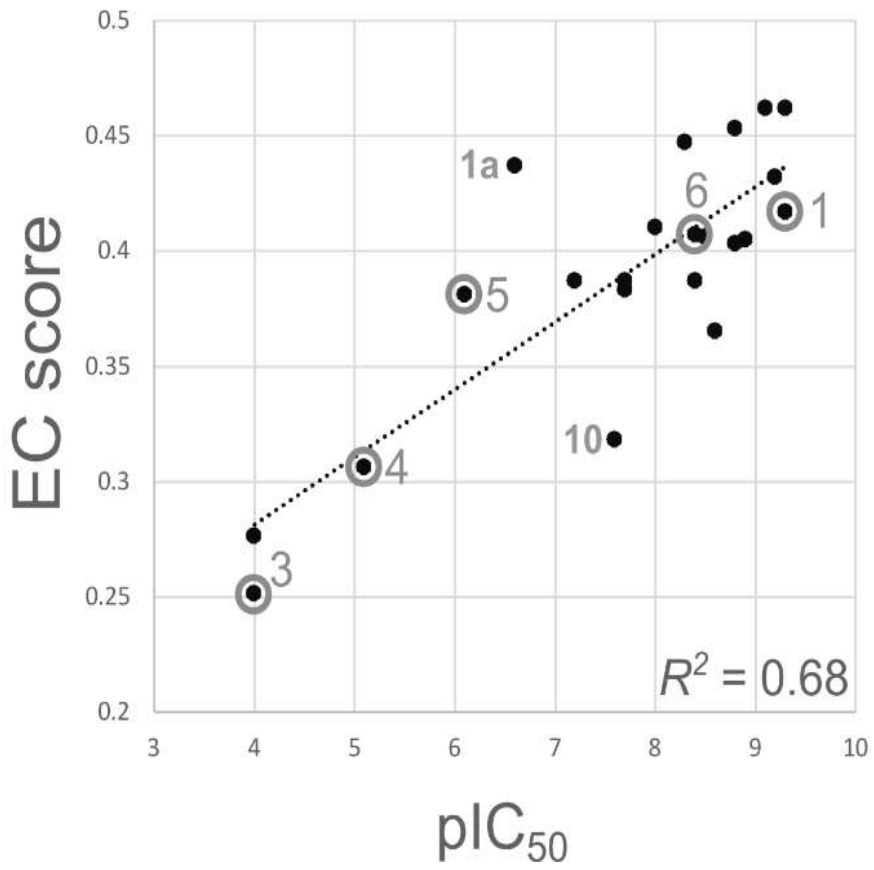


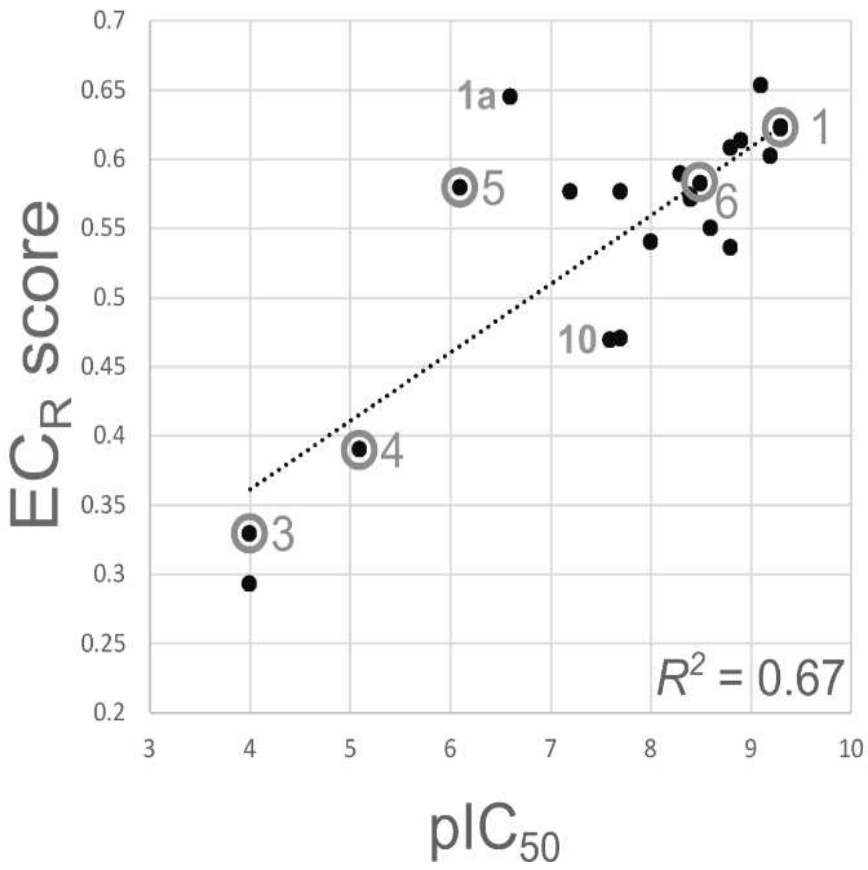
B



	Compound 13	Compound 17	Compound 21	Compound 25
pKi =	8.4	7.7	8.5	9.3
SCI =	0.40	0.38	0.40	0.46
R =	0.57	0.47	0.58	0.62
Rho =	0.52	0.48	0.56	0.61

EC correlation with activity

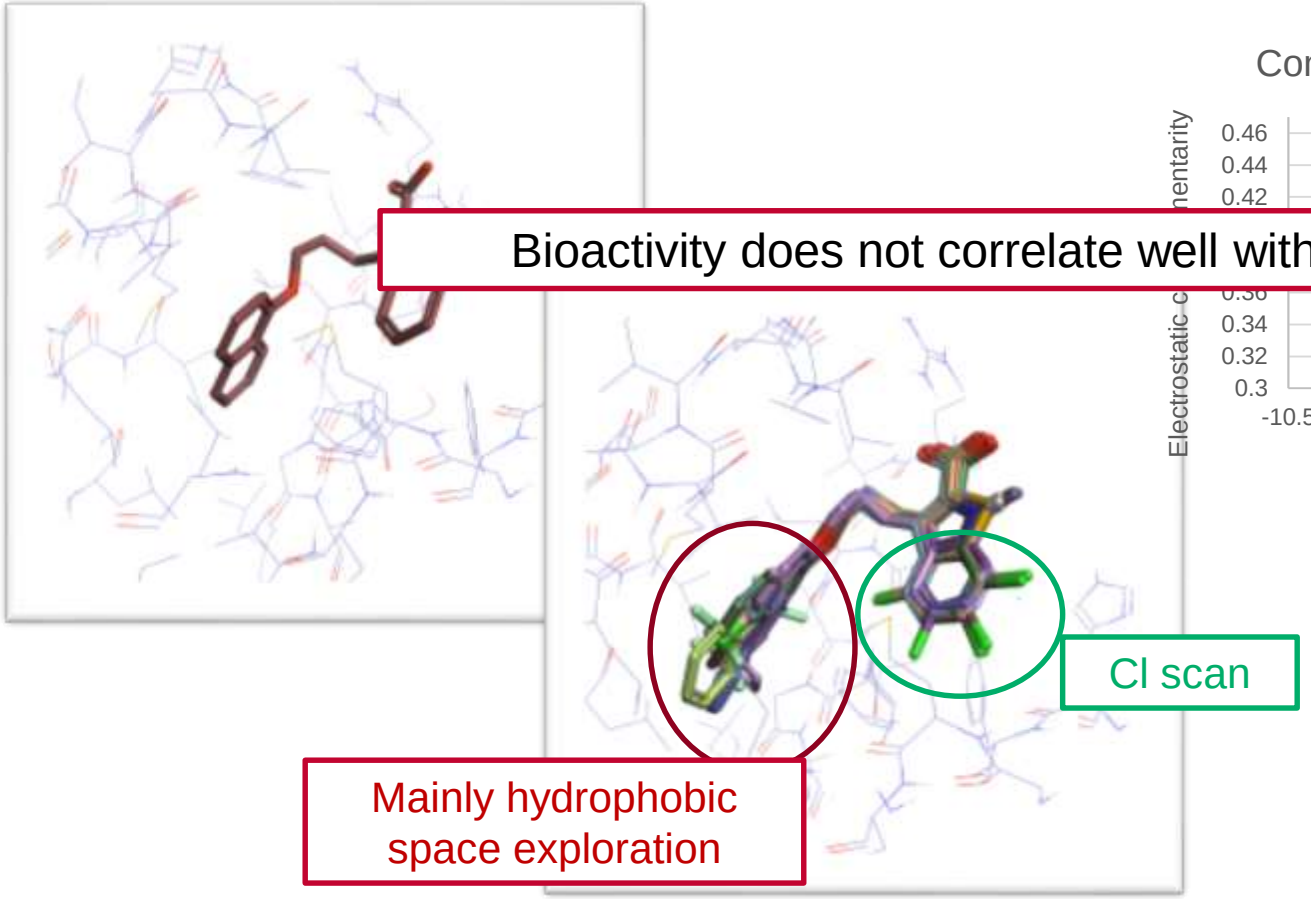


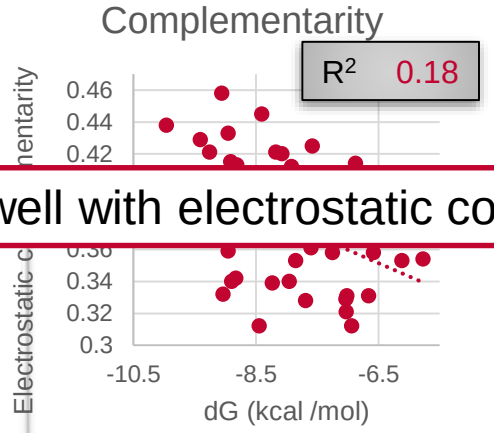


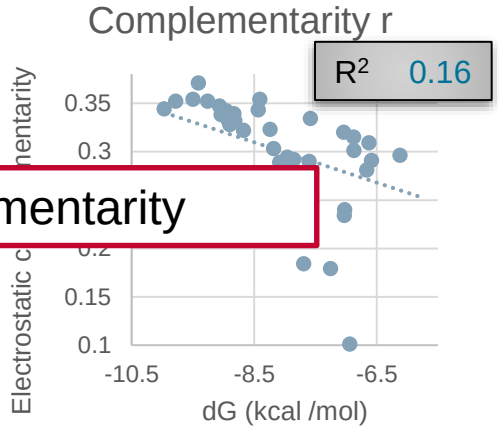
Application to MCL1

> MCL1 data set part of Schrodinger FEP benchmark (Wang *et al.* JACS 2015)

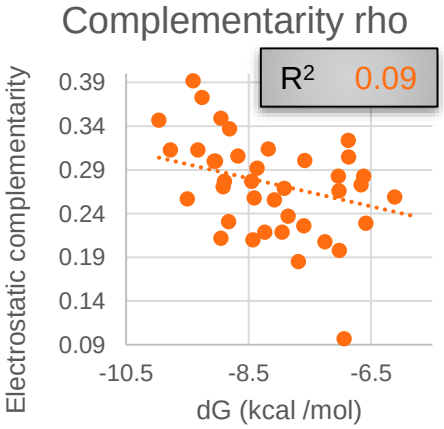
> 6B4L X-ray structure as receptor and X-ray ligand used as reference for Forge™ alignment





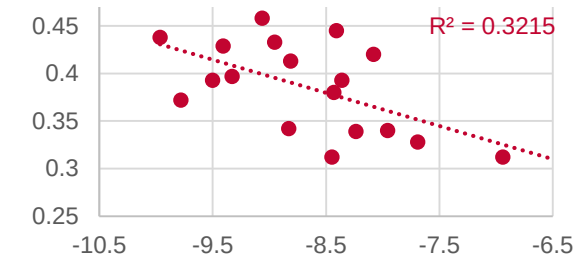


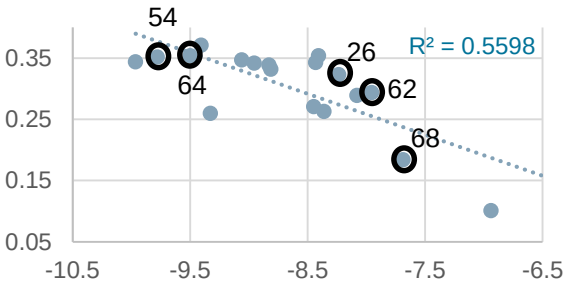
Bioactivity does not correlate well with electrostatic complementarity

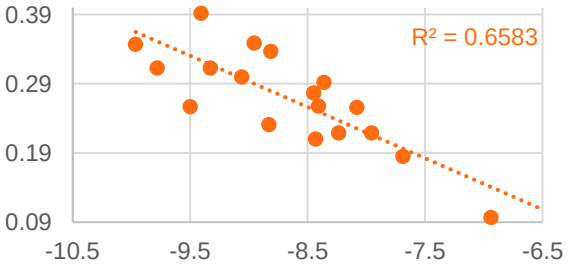


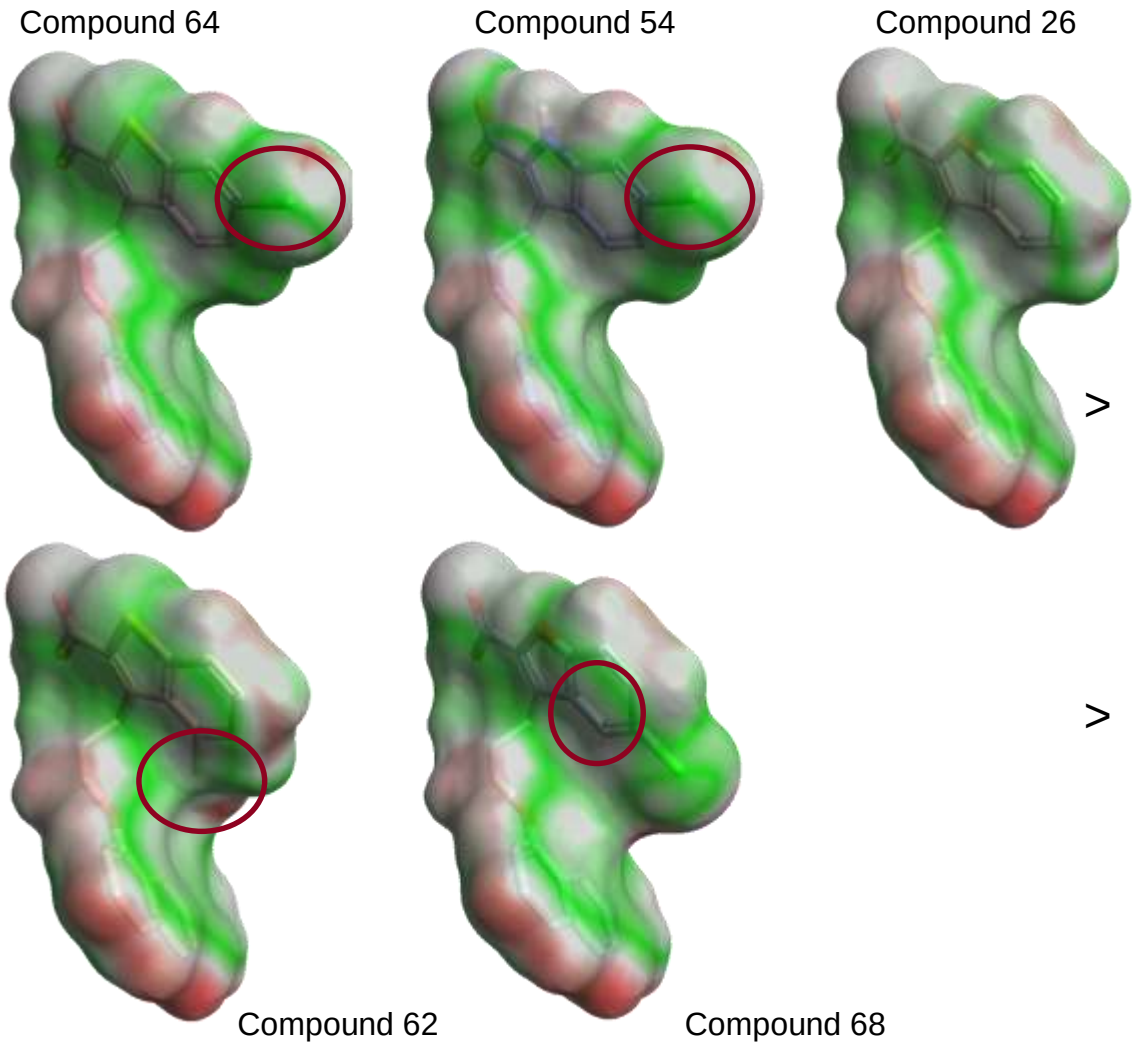
MCL1 – Cl scan subset correlates with ΔG

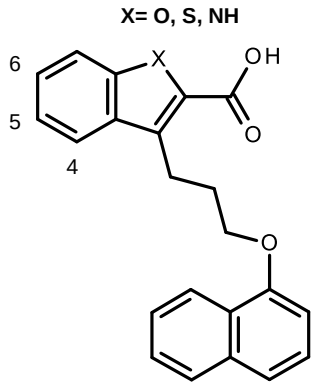
> Cl scan subset







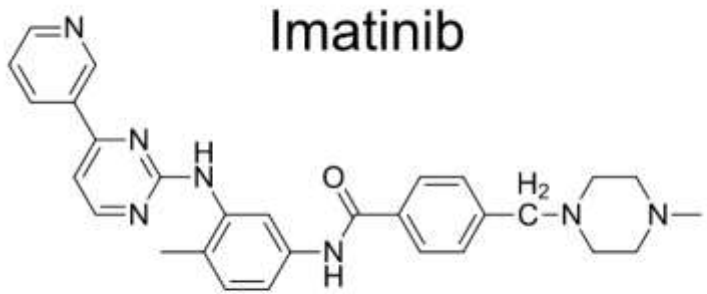




> 6-Cl substitution (64, 54) most favourable (good geometry for halogen bond with Ala227 BB)

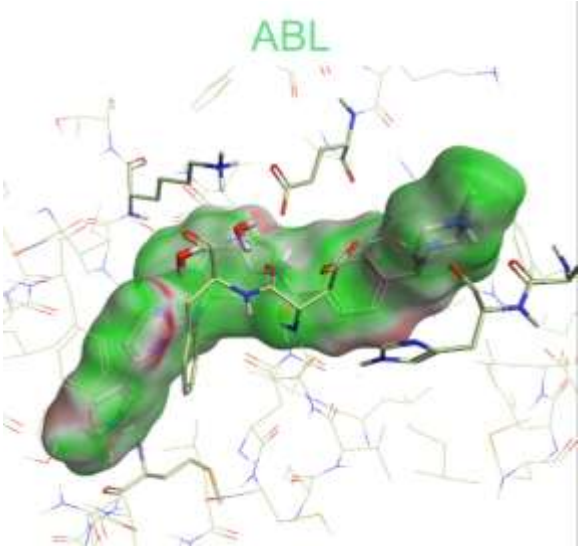
> 5-Cl (68) less favorable (electrostatic clash between the chlorine and the π-plane of Phe270)

Imatinib – EC and selectivity

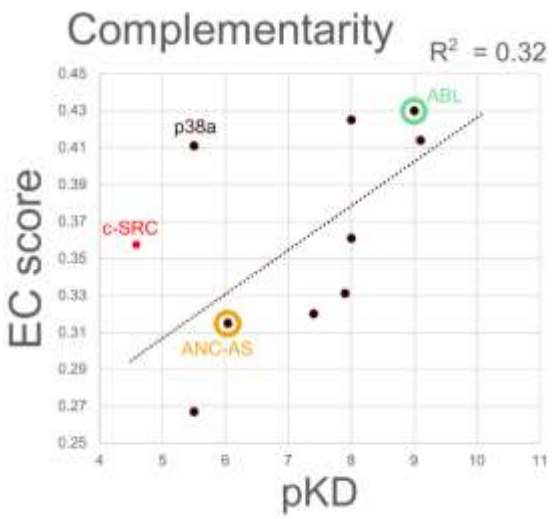


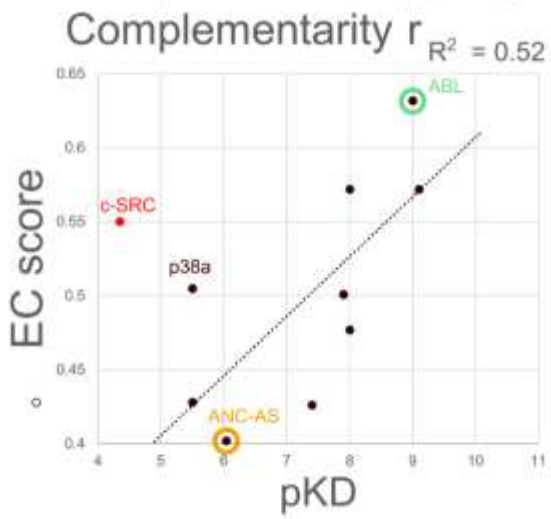
target	pdb	pKD	Complementarity	Complementarity r
c-SRC [a]	2OIQ	4.4	0.36	0.55
p38a	3HEC	5.5	0.41	0.51
SYK	1XBB	5.5	0.27	0.43
ANC-AS	4CSV	6.0	0.32	0.40
LCK	2PL0	7.4	0.32	0.43
KIT	1T46	7.9	0.33	0.50
CSF1	4R7I	8.0	0.36	0.48
ABL2	3GVU	8.0	0.43	0.57
ABL	1OPJ	9.0	0.43	0.63
DDR1	4BKJ	9.1	0.41	0.57

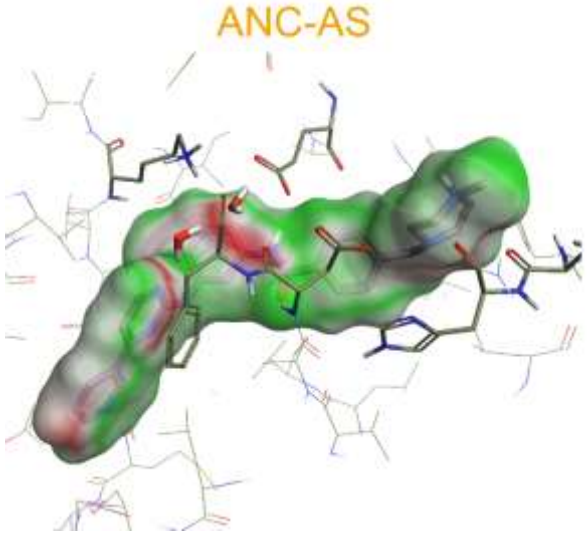
[a] Imatinib binding decreased due to conf. penalty upon binding



c-SRC binding induces conformational change of protein, and c-SRC and p38 binding are highly solvent-exposed.

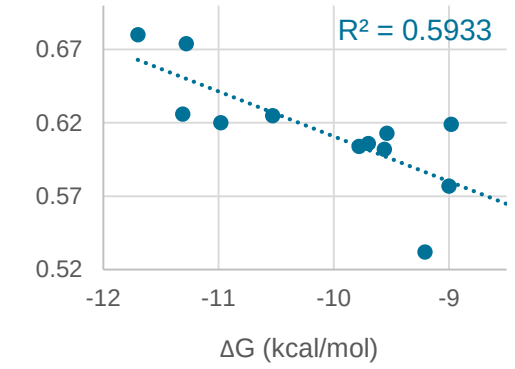




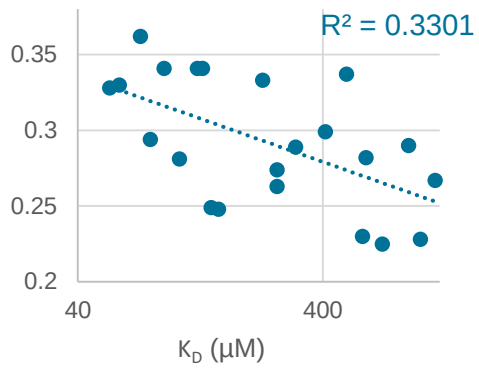


Application to additional data sets

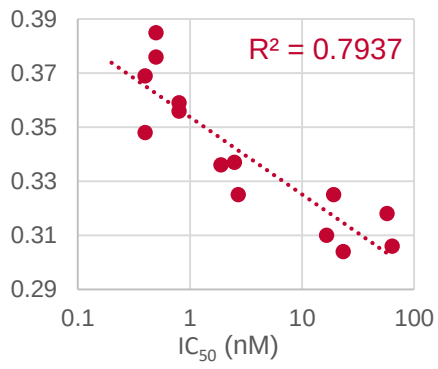
TYK2



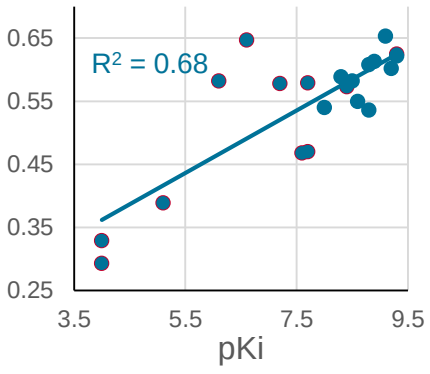
RPA70N



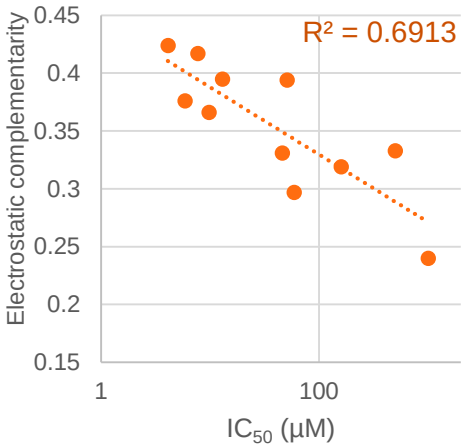
PERK



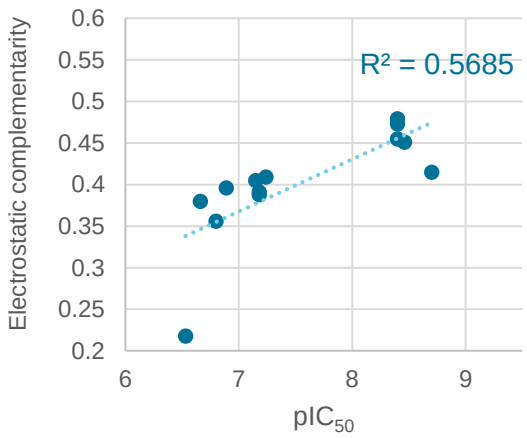
mGlu5



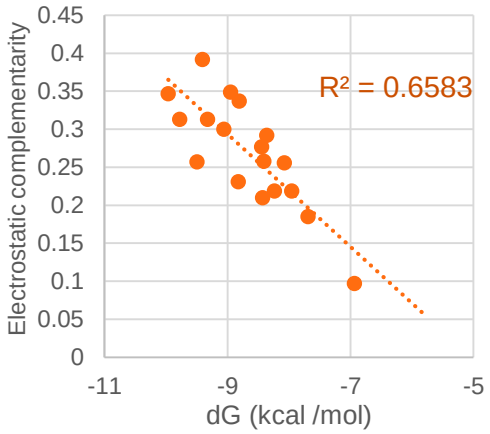
XIAP



DPP4

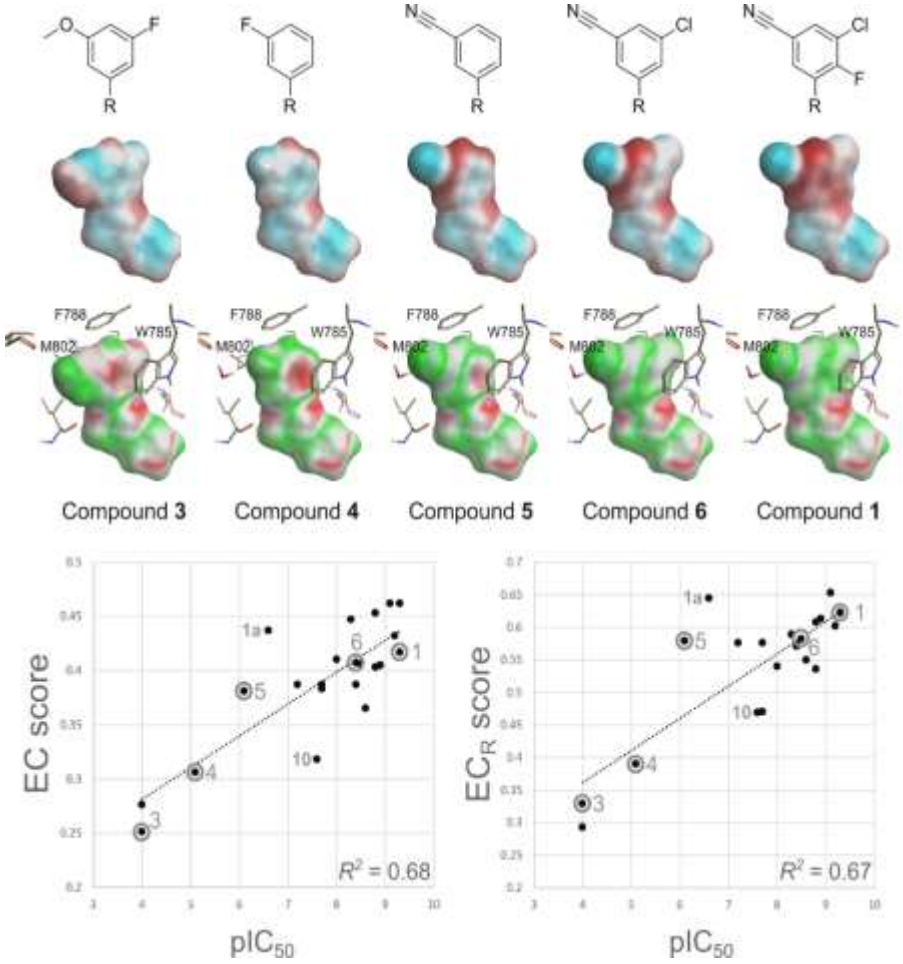


MCL1

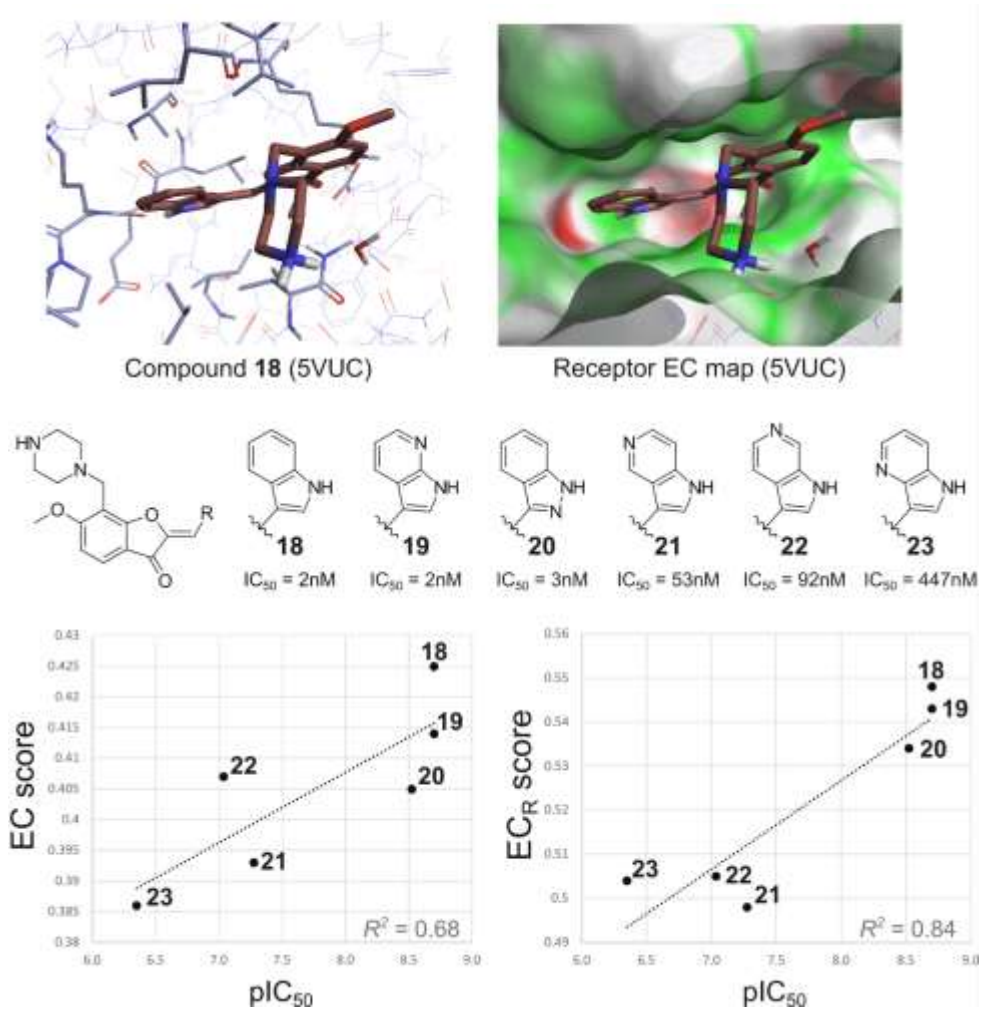


Complementarity; Complementarity r; Complementarity rho

Plus more:



mGLU5



PIM-1

Comparison to QM

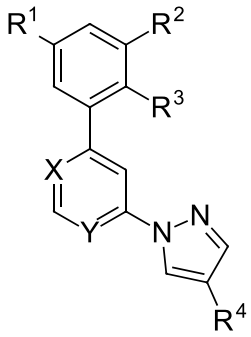
> Is the XED force field giving good enough results?

> Can we compute EC scores at the QM level?

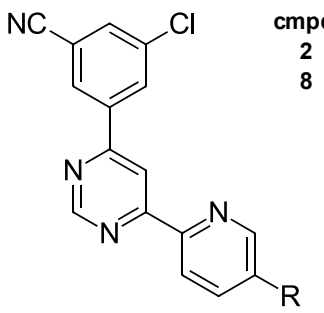
Truncated mGLU5 example (5CGC)

- Truncated binding site mode of 5CGC
- → Corresponds to more or less 6Å binding site definition in Flare
- → no formal charges
- → analysis of 12 ligands (table 1)

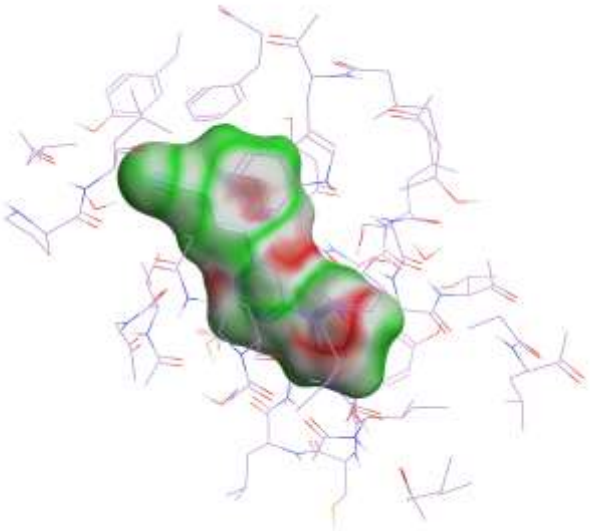
*Christopher et al, **J. Med. Chem.** 2015*



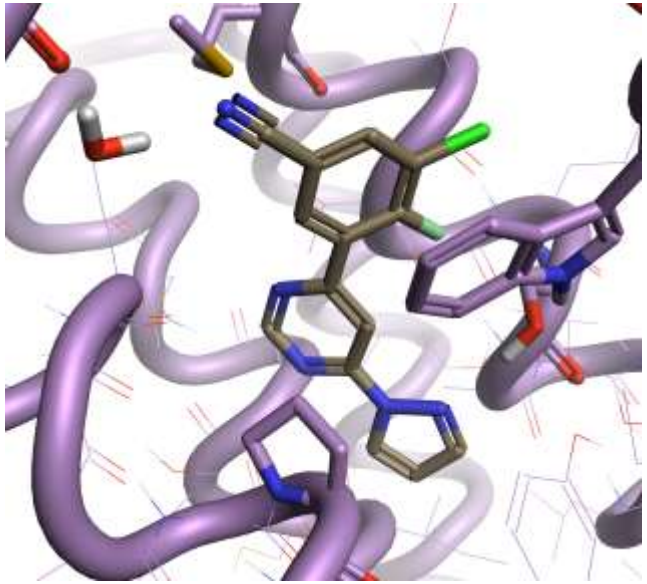
cmpd	X	Y	R^1	R^2	R^3	R^4
1	N	N	CN	Cl	F	H
1a	N	N	CN	H	F	H
3	N	N	OMe	F	H	H
4	N	N	F	H	H	H
5	N	N	CN	H	H	H
6	N	N	CN	Cl	H	H
7	N	N	CN	Cl	H	F
9	N	N	CN	Me	H	H
10	N	CH	CN	Me	H	H
11	CH	N	CN	Me	H	H

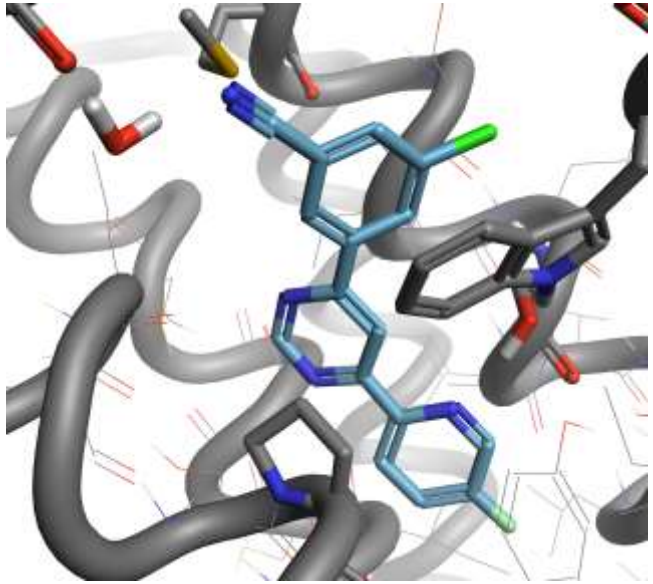


cmpd	R
2	F
8	H



Truncated 5CGC with EC map (**5**)

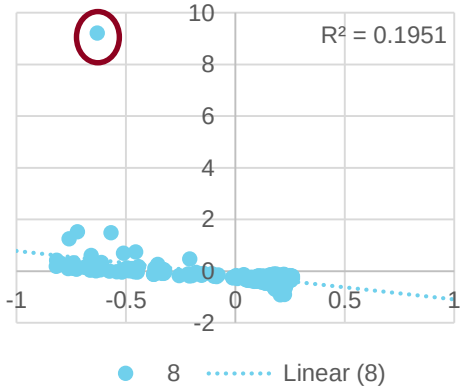


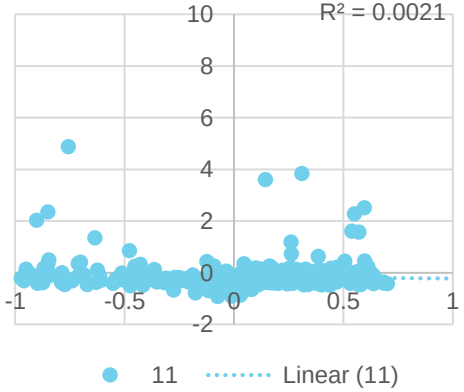


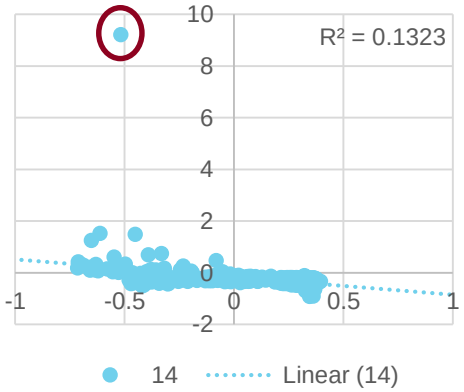
ESP value outliers

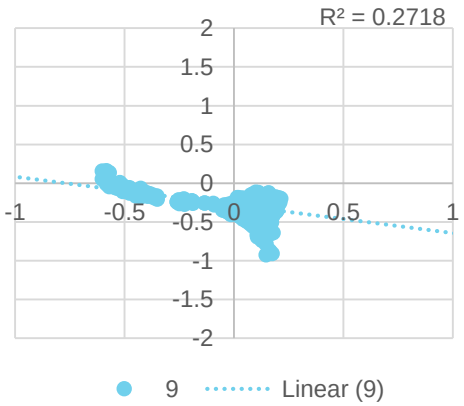
Outlier at the tip of –CN substituent for protein potential (water molecule too close?!)

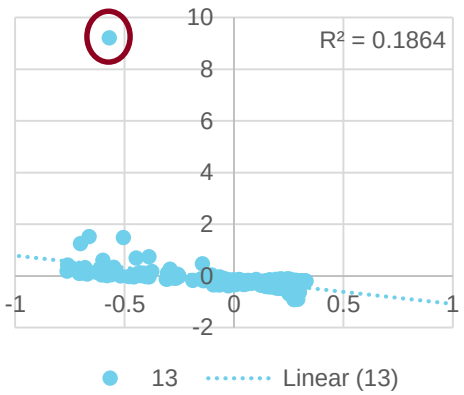
Outlier excluded from further analysis





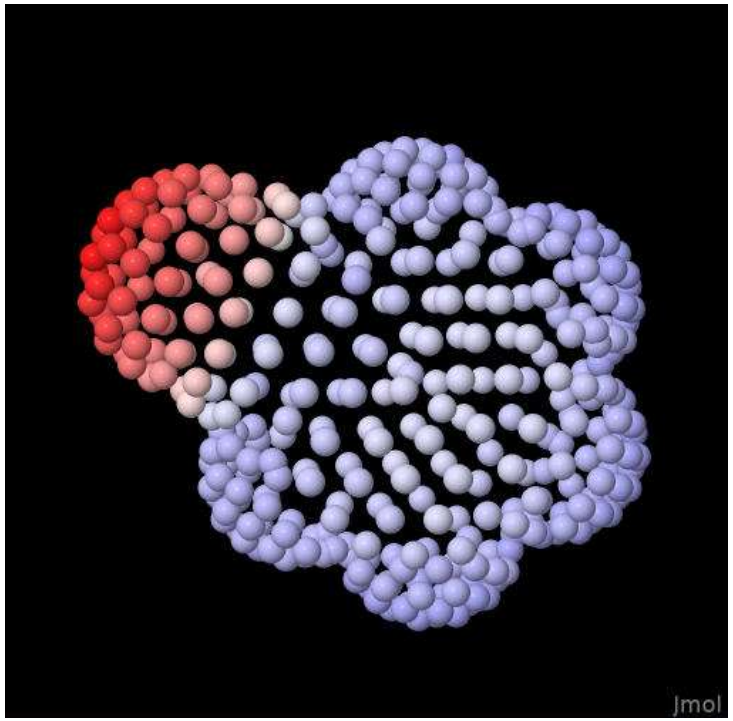




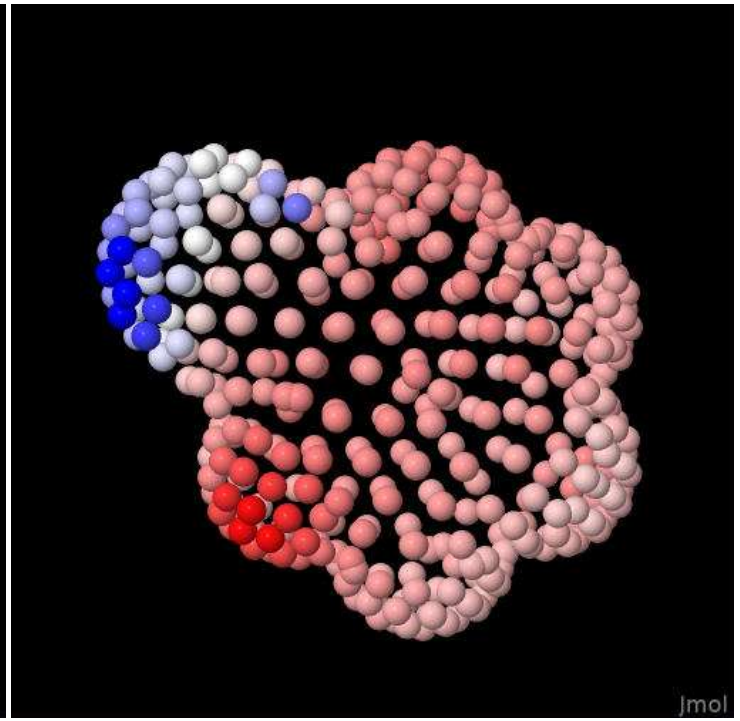


Outlier excluded from further analysis

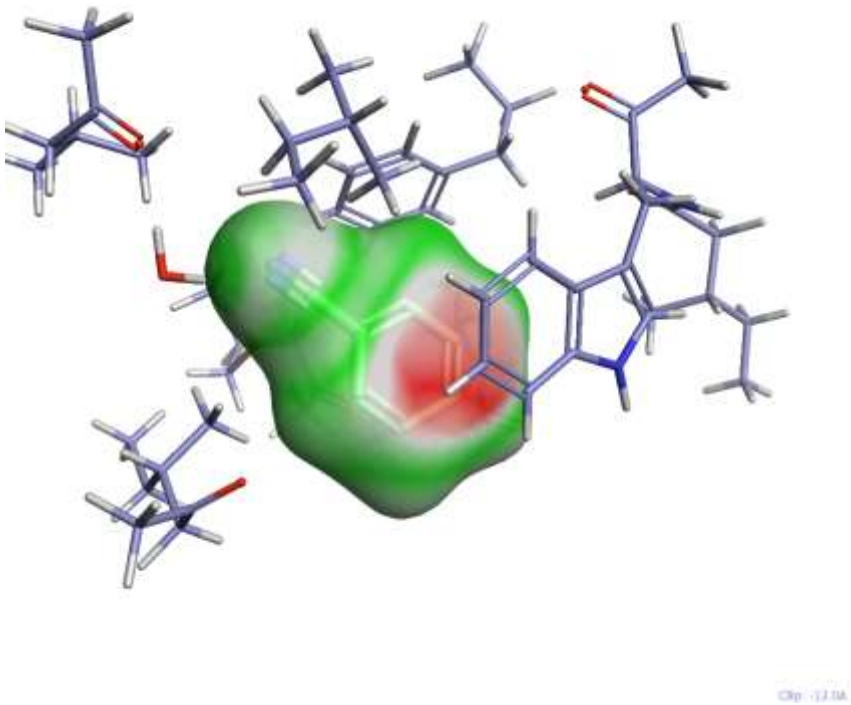
ESP value outliers



Cmpd 8 – Ligand ESP



Cmpd 8 – Protein ESP



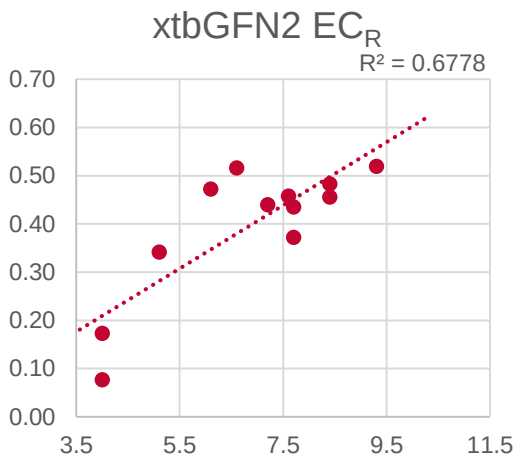
H of water molecule
very close to CN group

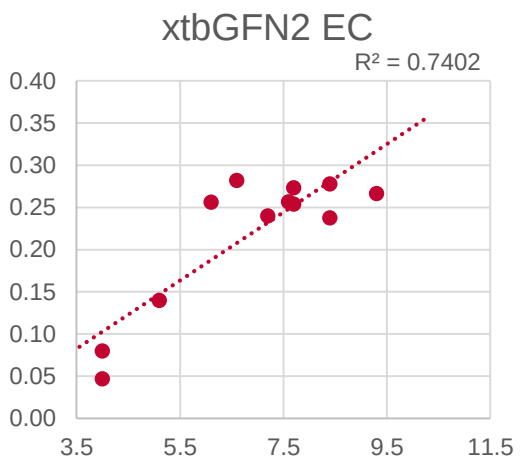
It is not just important HOW you calculate the electrostatic potential but also WHERE

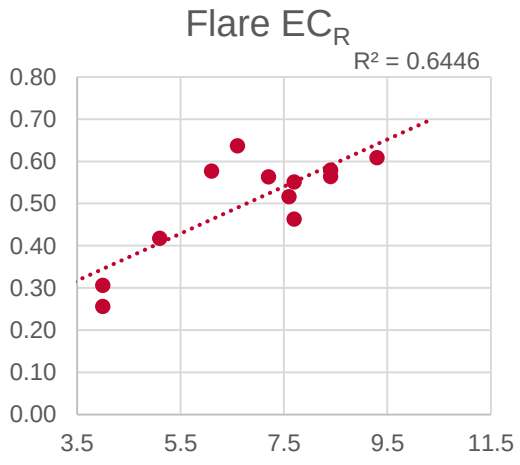
Truncated mGLU5 example (5CGC) - Flare vs XTB EC correlation

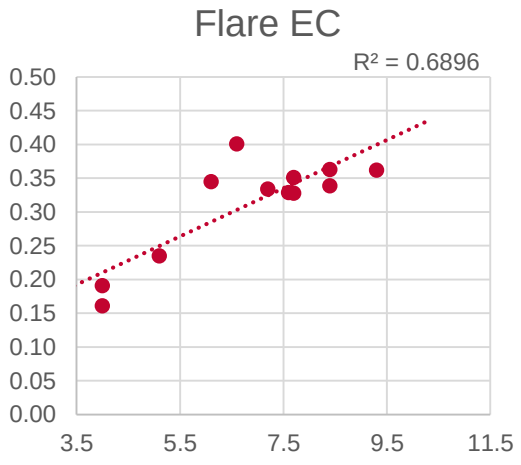
- xtbGFN2 and XED (Flare) are similarly predictive
- Use of truncated 5CGC binding pocket and table 1 data (12 ligands)
- Protein ESP outliers for xtbGFN2 (ESP values over 5) were excluded

	pIC50	Flare EC_R	GFN2 EC_R	Flare EC	GFN2 EC
10	4	0.26	0.17	0.16	0.08
11	4	0.31	0.08	0.19	0.05
12	8.4	0.58	0.46	0.36	0.28
13	8.4	0.56	0.48	0.34	0.24
14	9.3	0.61	0.52	0.36	0.27
15	7.6	0.52	0.46	0.33	0.26
16	7.7	0.55	0.44	0.35	0.27
17	7.7	0.46	0.37	0.33	0.25
6	7.2	0.56	0.44	0.33	0.24
7	6.6	0.64	0.52	0.40	0.28
8	6.1	0.58	0.47	0.35	0.26
9	5.1	0.42	0.34	0.24	0.14

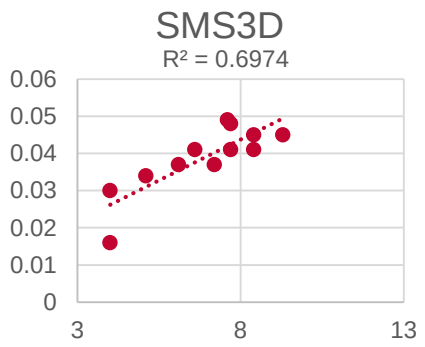


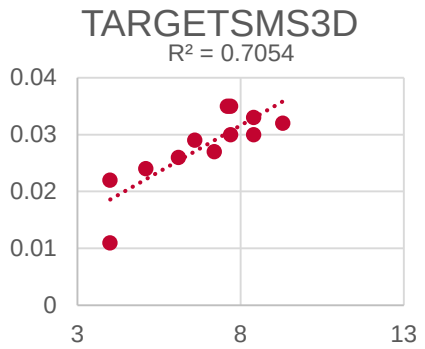


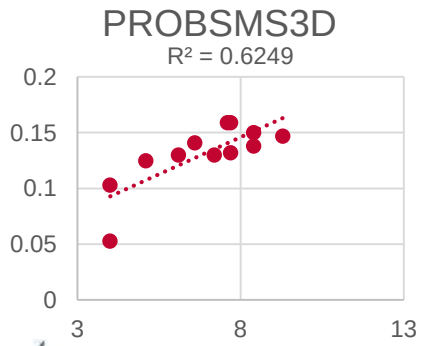




Truncated mGLU5 example (5CGC) – COSMOsim3D



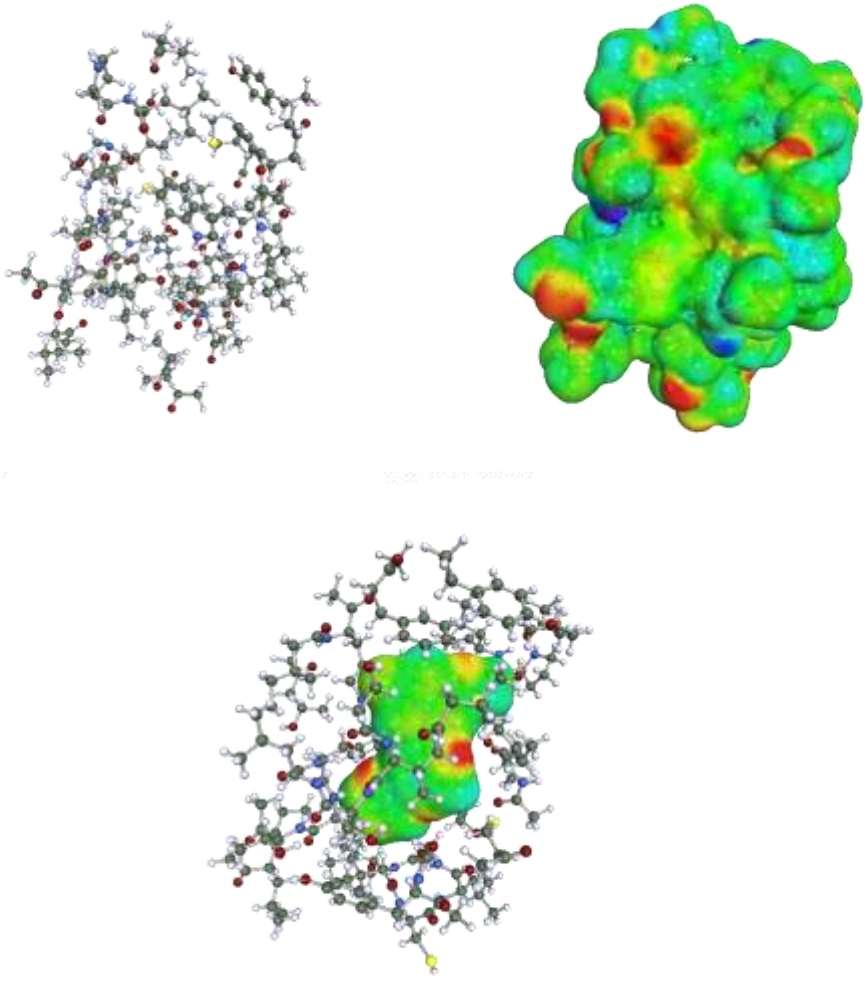




- Calculation of COSMO surfaces with Turbomole (BLYP-D3-SVP level for ligands and HF3c-D3 for receptor)

- Experimental function of COSMOsim3D can calculate similarity between inverse receptor surface and ligand COSMO surfaces

→ good correlation, but takes several hours to compute cosmo surface for truncated receptor (7-8h at HF3c level with TURBOMOLE on a workstation)

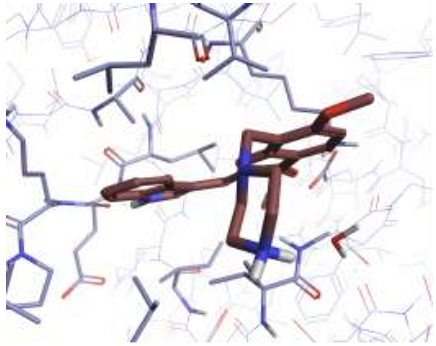


Truncated 5VUC (PIM1) example

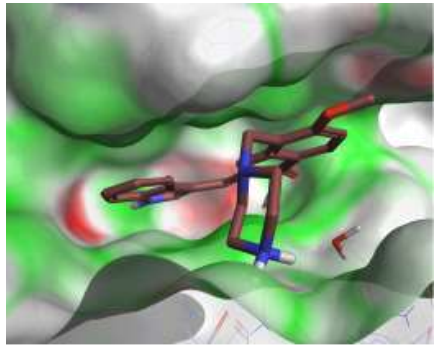
- 6 ligands with N scan on indole moiety (literature data set that was used for FMO analysis and binding energy correlation)

  *Watanabe et al., **JCIM** 2017*

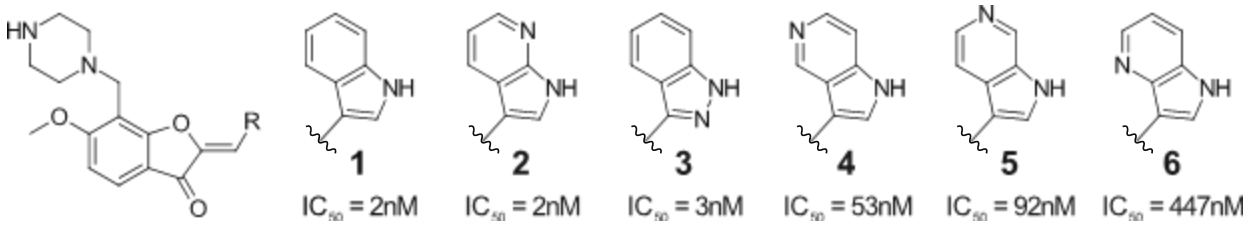
- Both ligand and protein heavily charged, although not in close proximity to indole moiety

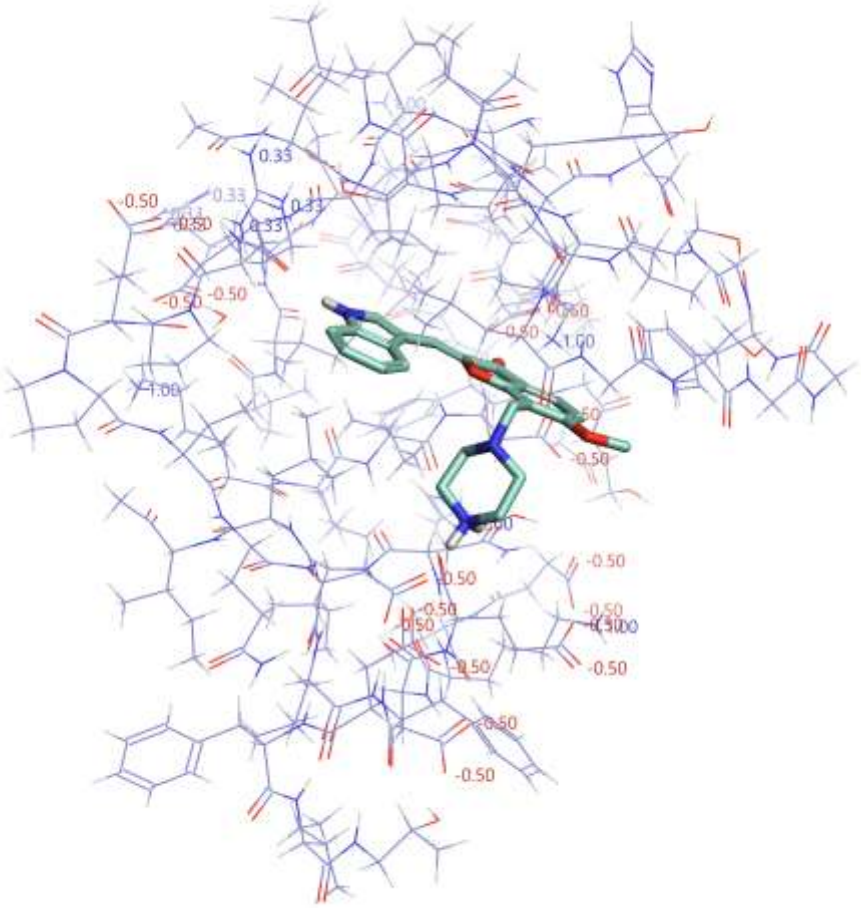


Compound **1** (5VUC)



Receptor EC map (5VUC)



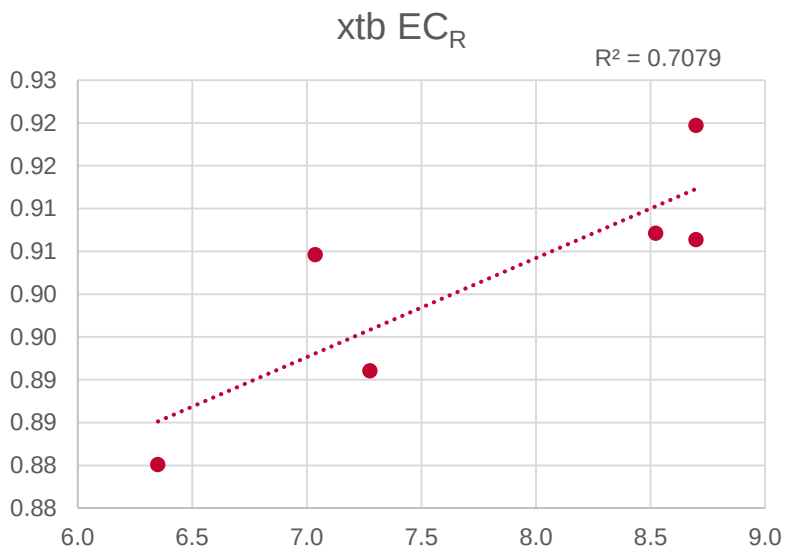


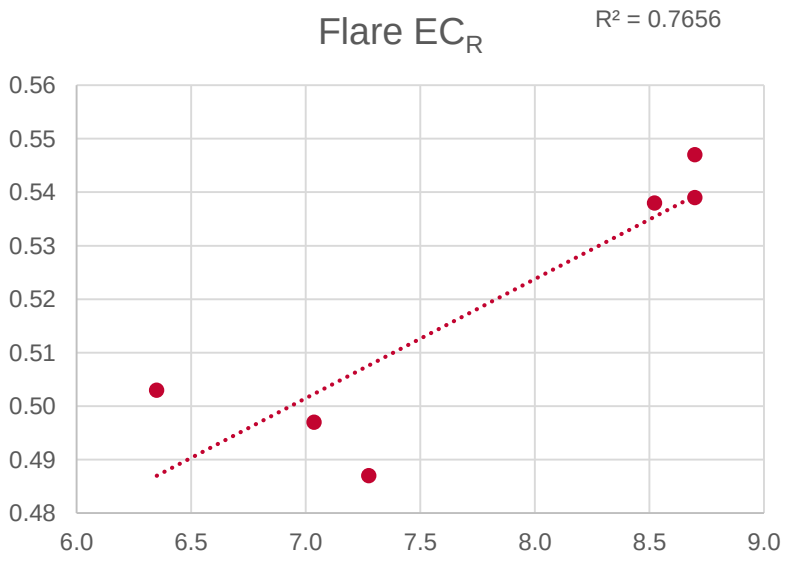
Truncated 5VUC with **1**

Truncated 5VUC (PIM1) example – with GBSA

	xtb EC_R	IC50	pIC50
1	0.91	2.00E-09	8.7
2	0.92	2.00E-09	8.7
3	0.91	3.00E-09	8.5
4	0.89	5.30E-08	7.3
5	0.90	9.20E-08	7.0
6	0.88	4.47E-07	6.3

	Flare EC	Flare EC_R	IC50	pIC50
1	0.428	0.55	2.00E-09	8.7
2	0.414	0.54	2.00E-09	8.7
3	0.4	0.54	3.00E-09	8.5
4	0.406	0.49	5.30E-08	7.3
5	0.419	0.50	9.20E-08	7.0
6	0.393	0.50	4.47E-07	6.3

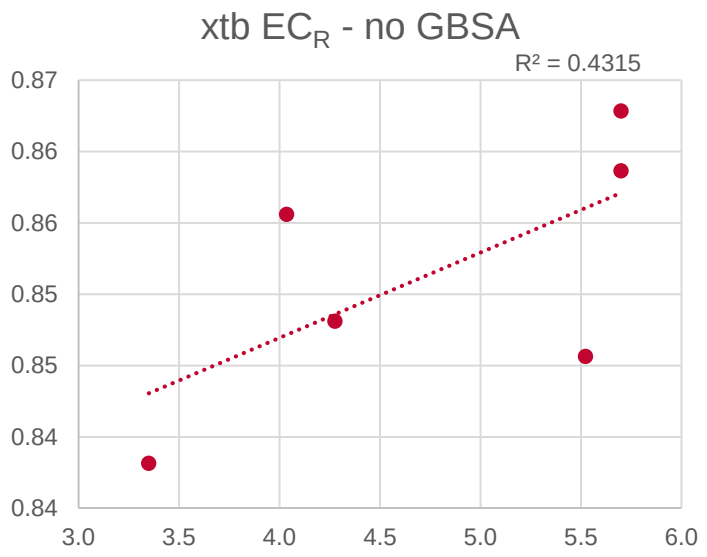


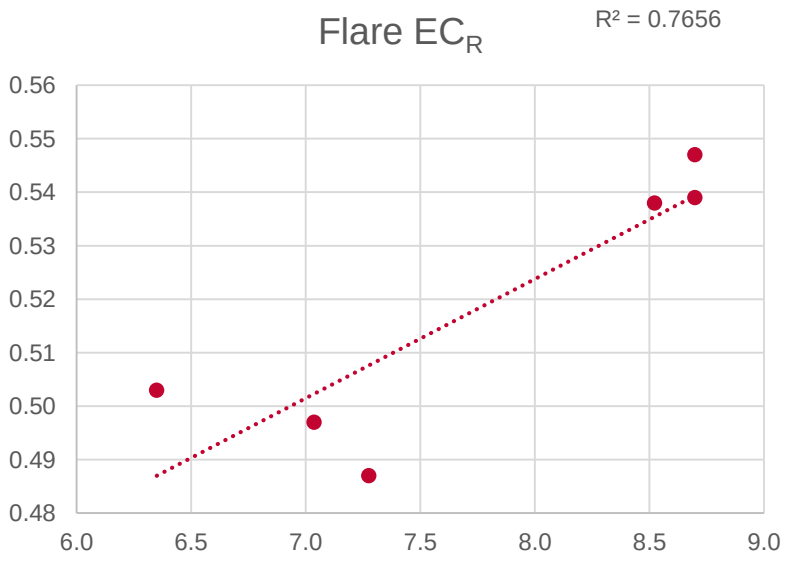


Truncated 5VUC (PIM1) example – no GBSA

	xtb EC_R - no GBSA	IC50	pIC50
1	0.86	2.00E-09	8.7
2	0.86	2.00E-09	8.7
3	0.85	3.00E-09	8.5
4	0.85	5.30E-08	7.3
5	0.86	9.20E-08	7.0
6	0.84	4.47E-07	6.3

	Flare EC	Flare EC_R	IC50	pIC50
1	0.428	0.55	2.00E-09	8.7
2	0.414	0.54	2.00E-09	8.7
3	0.4	0.54	3.00E-09	8.5
4	0.406	0.49	5.30E-08	7.3
5	0.419	0.50	9.20E-08	7.0
6	0.393	0.50	4.47E-07	6.3





Results become much worse without GBSA!

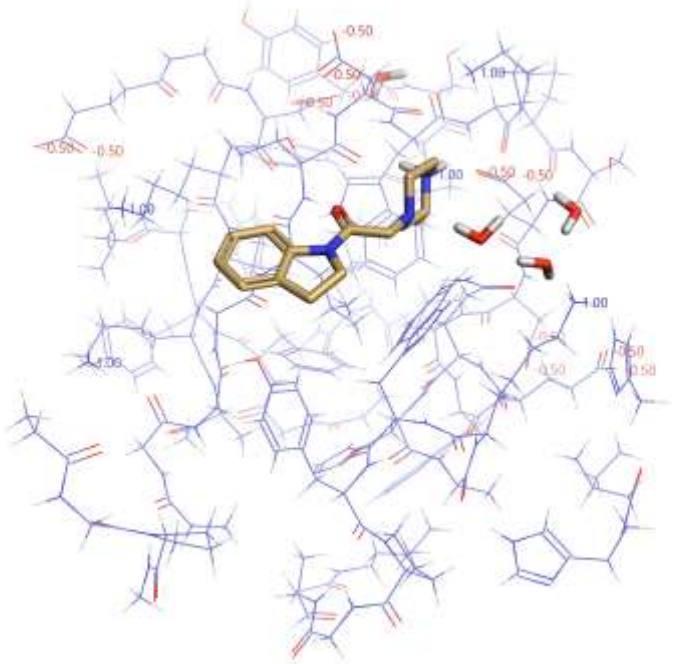
Truncated XIAP (5C7D) example

- PPI target with inhibitors that show electrostatic SAR

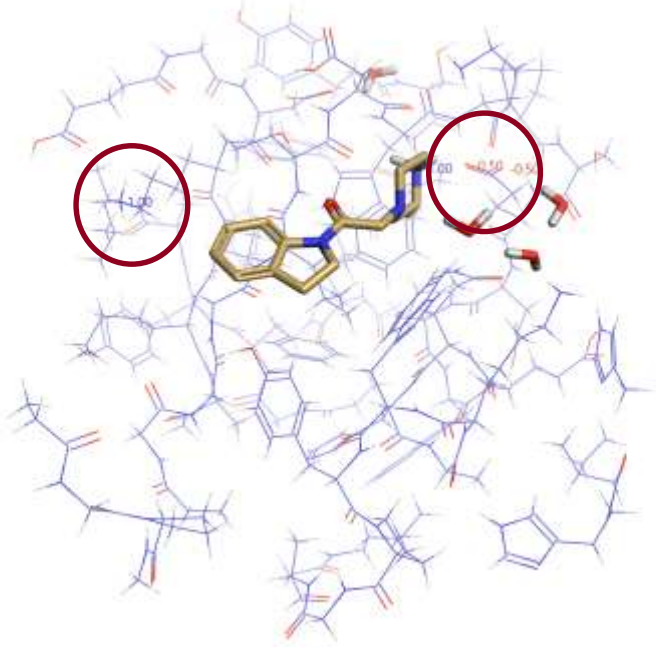
  Chessari et al., ***J. Med. Chem*** *2015*

- Binding site exhibits a large number of formal charges

- Preparation of charged and ‘neutral’ receptor



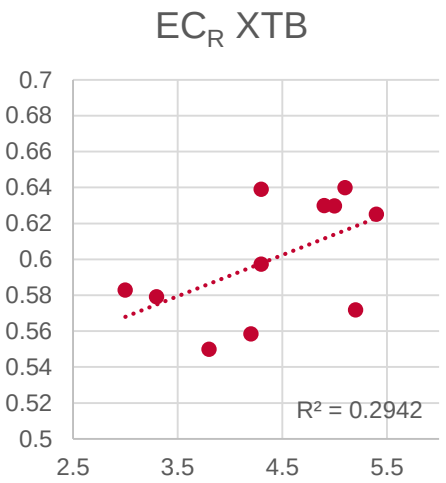
charged

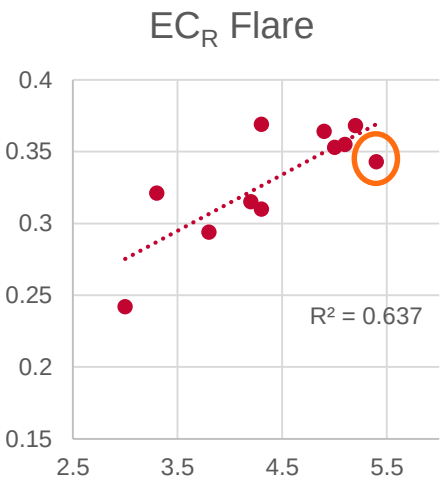


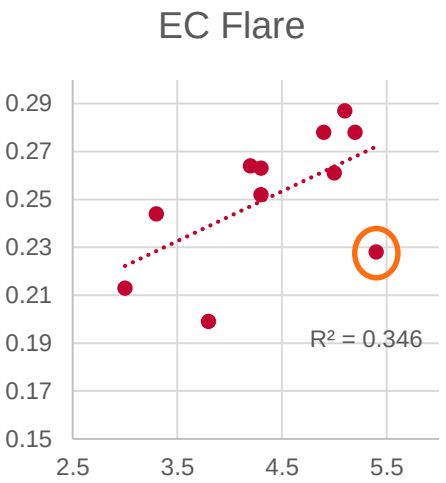
‘neutral’
2 important (close contact to ligand) charges left

Truncated XIAP example – EC correlation

charged

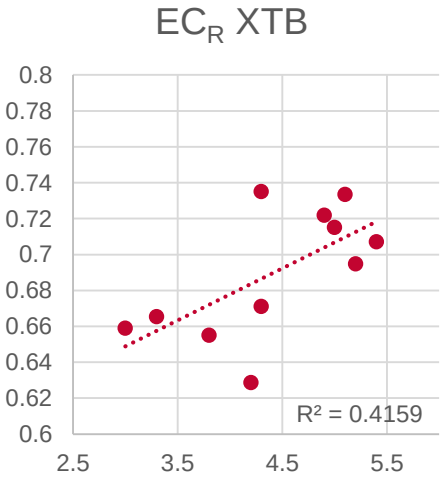


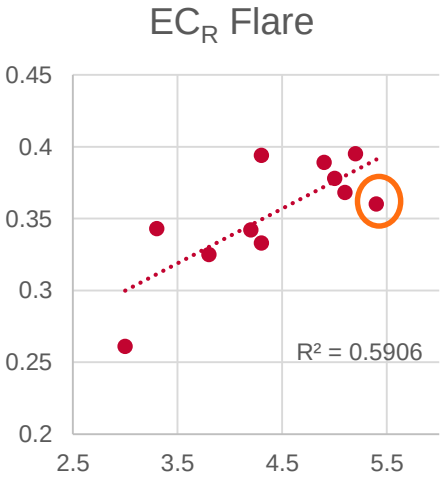


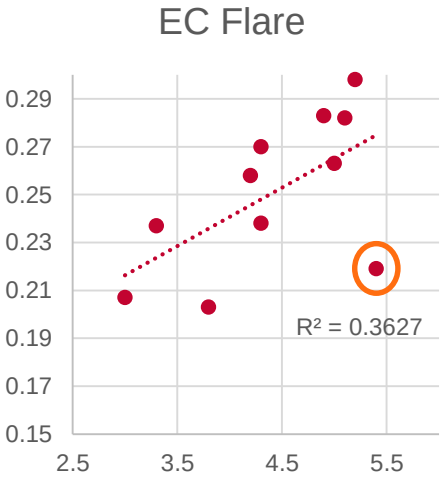


Compound 16 weak clash with Lys sc

'neutral'
2 important charges left

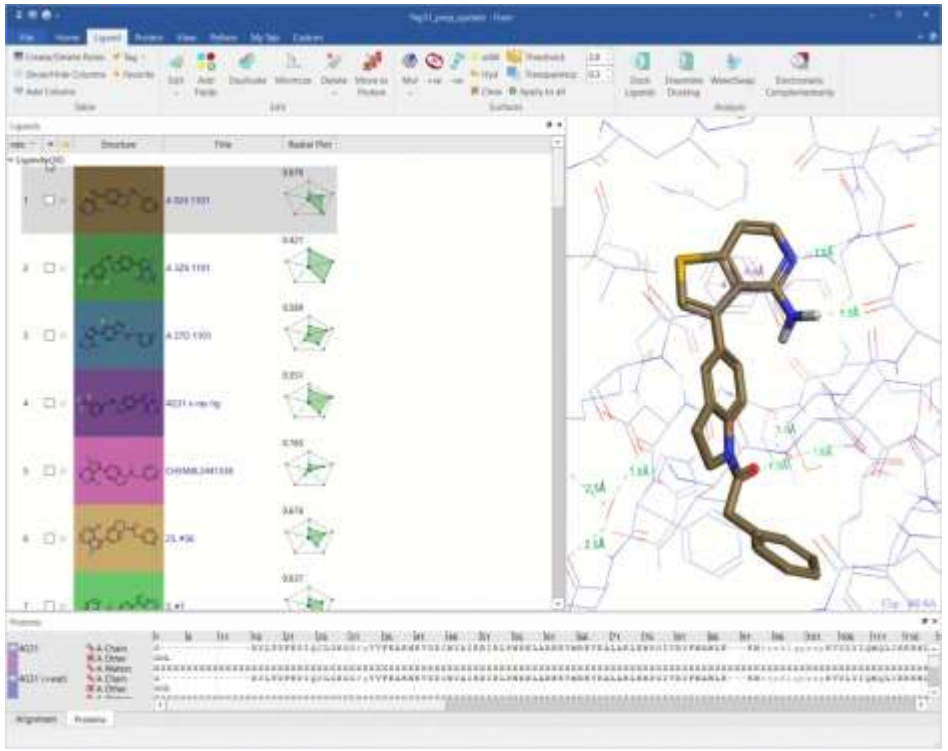






Conclusion and Outlook

- Meaningful assessment of electrostatic complementarity at low computational costs (< 1 second per molecule on a desktop workstation)
- Possible to rank bioactivities of ligands (provided electrostatics play a main role in affinity changes)
- Caveats: does not calculate free energy of binding ΔG (desolvation, cavity term and space filling, entropic contributions, conformational effects missing); orthogonal multipolar interactions (fluorine bonding)
- Additional validation and future research: Improved handling of solvent exposed areas, rescoring of docking results, further evaluation of and comparison to *ab initio* approaches



Try EC on your dataset using Flare:
http://cresset-group.com/flare

cresset software

innovative science • intuitive software

Acknowledgements: **Matthias Bauer**
Paolo Tosco
Giovanna Tedesco
Andy Vinter

Cresset team (summer 2018)

Questions welcomed

mark@cresset-group.com

cressetgroup

cresset software

innovative science • intuitive software

mark@cresset-group.com

Request a free evaluation of Flare to try EC for yourself
https://www.cresset-group.com/evaluation-request/

cressetgroup