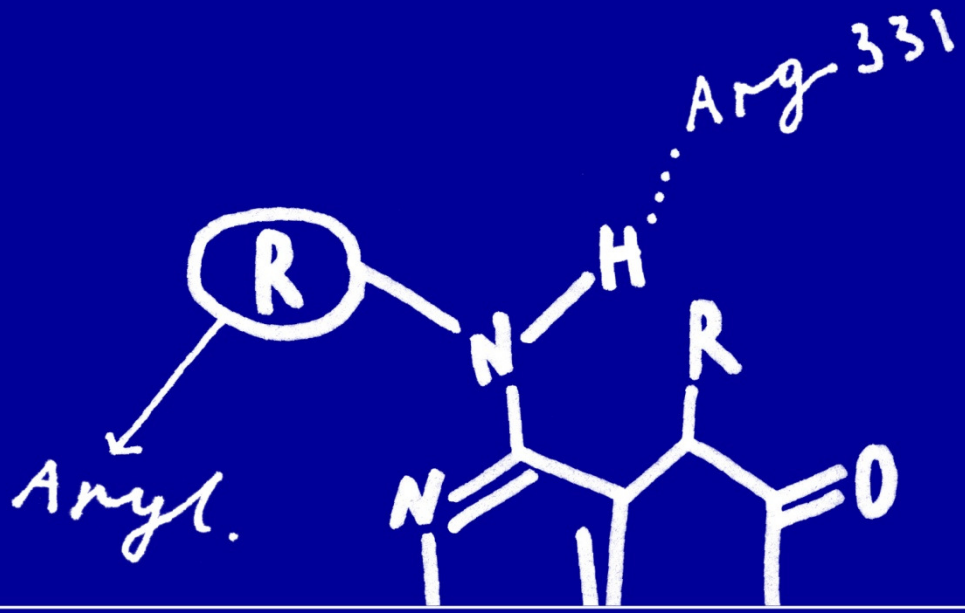
evotec

'RESEARCH NEVER STOPS'
Building innovative drug discovery alliances

Structure-based design for Kinases guided by FMO and water analysis

Is FMO a tool for prediction, analysis, or education?

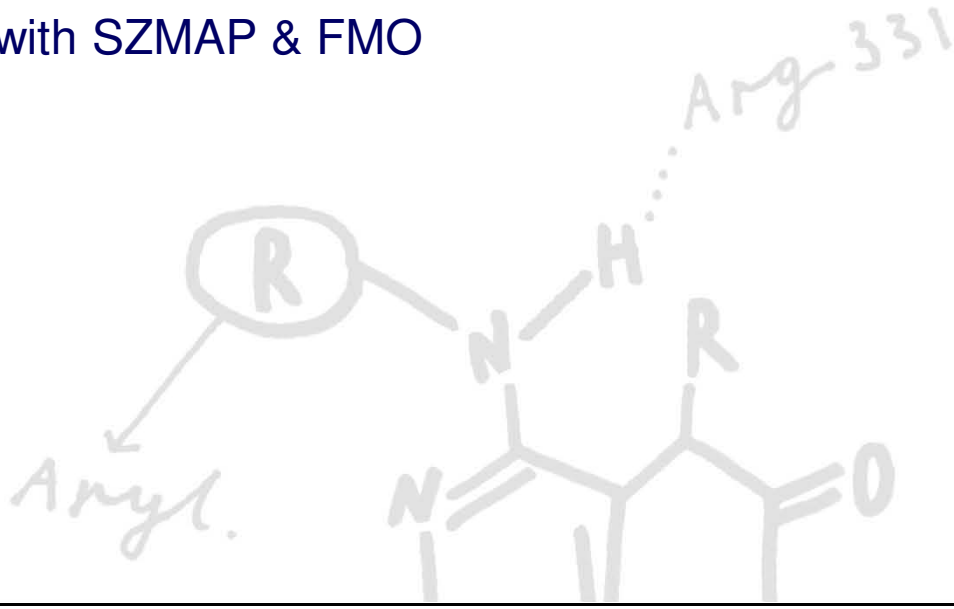
Richard J. Law



evotec

FMO for SBDD

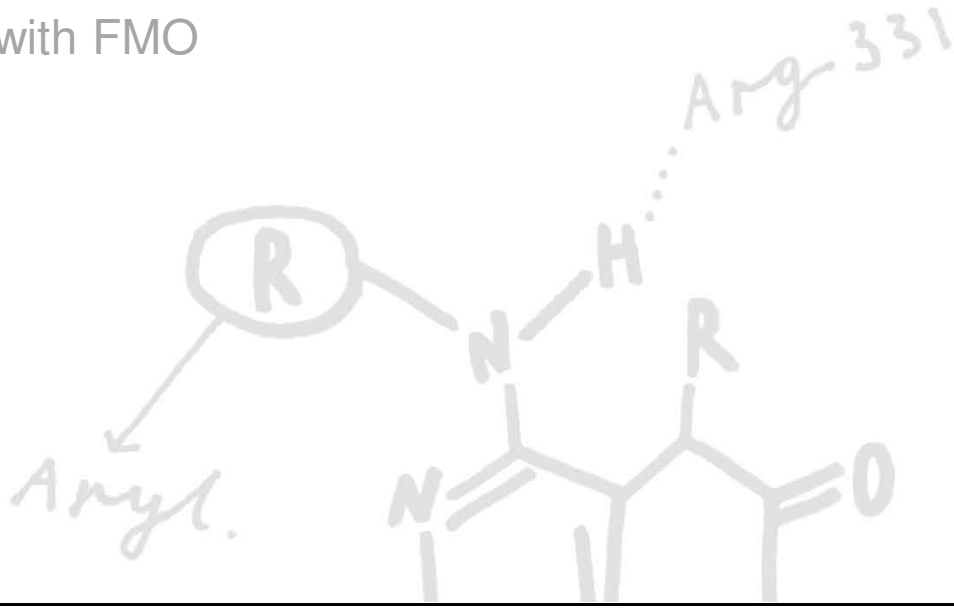
- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with SZMAP & FMO



evotec

FMO for SBDD

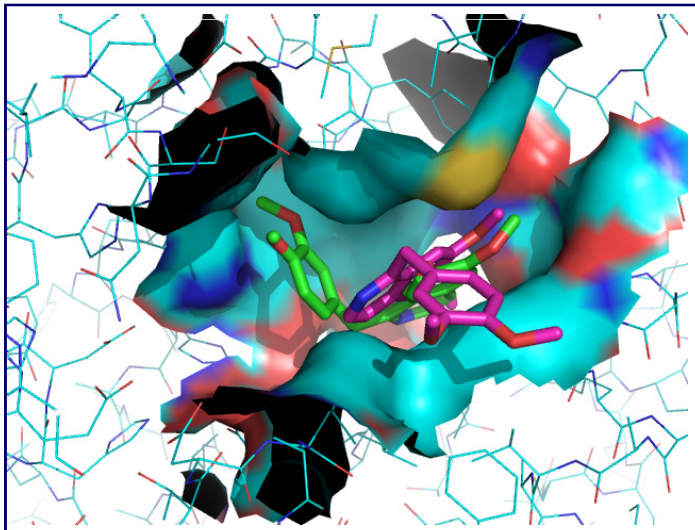
- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with FMO



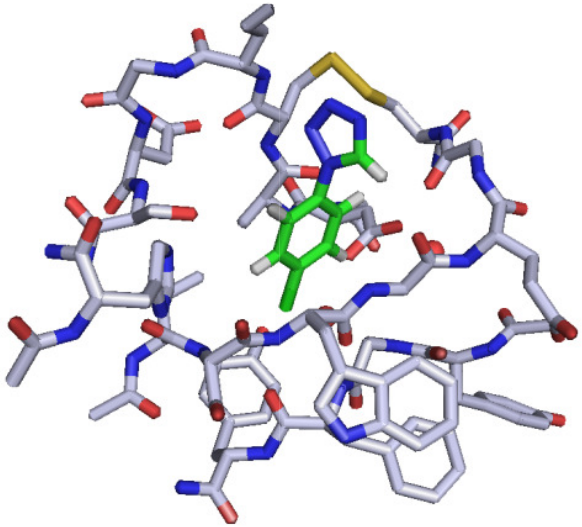
Understanding complex interactions

Guiding H2L/F2L/LO with precise SBDD

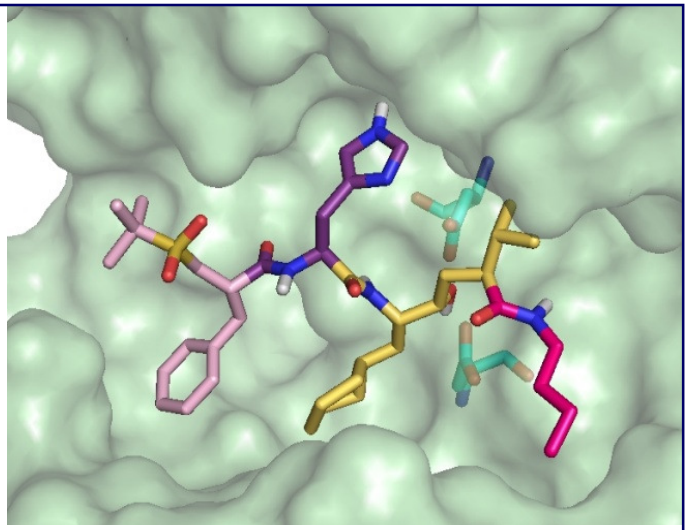
What happens when you need a better than MM understanding of your system?



Multiple equivalent binding modes



Interactions not represented in docking/MM forcefields

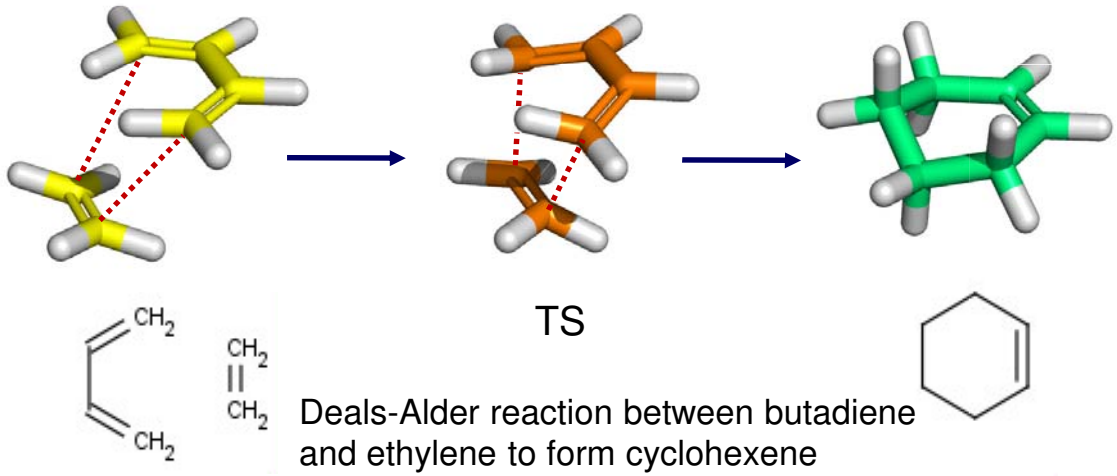


"Defragmentation" of large ligands to determine efficiency

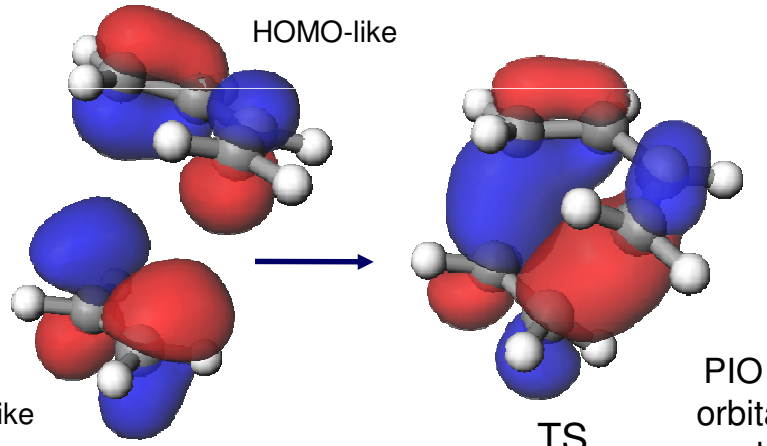
More complex methods required – e.g. electrostatics complementarity, free energy and/or **quantum mechanical calculations, e.g. FMO**

MM vs. QM; describing intermolecular interactions

Intermolecular forces have some elements of chemical bonding

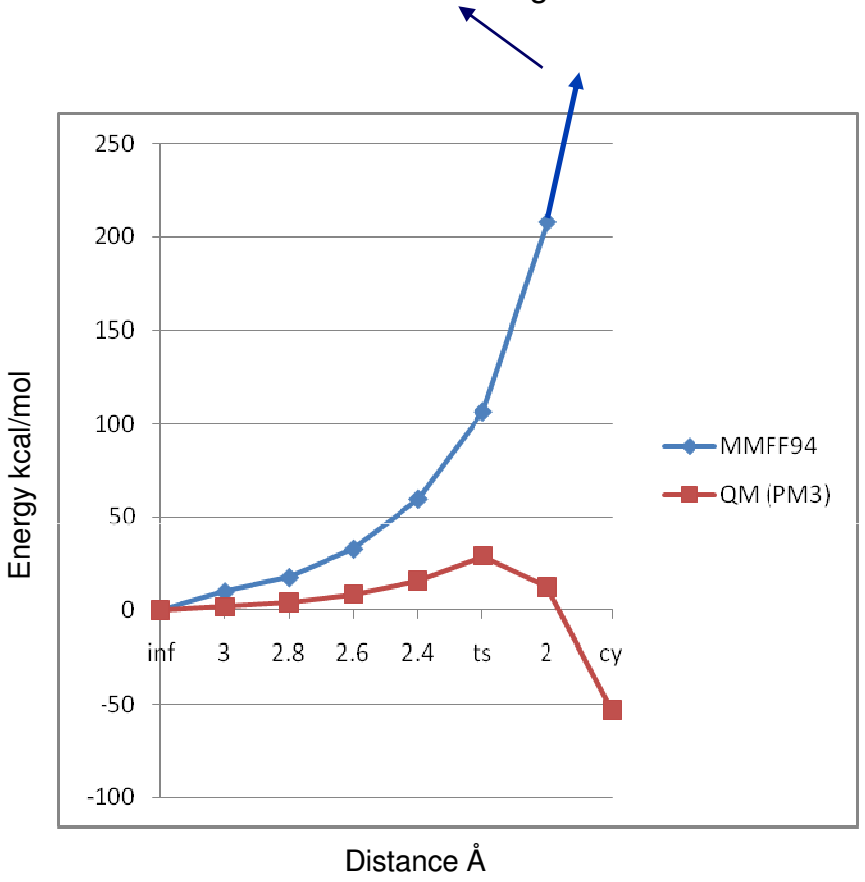


Deals-Alder reaction between butadiene and ethylene to form cyclohexene



PIO (paired interacting orbitals) visualisation of molecular interactions

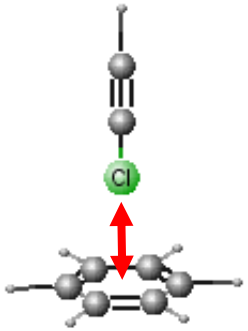
MM does not understand chemical bonds are forming

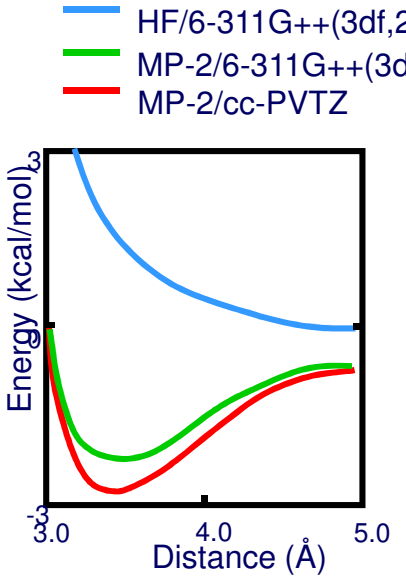


Observing protein::ligand complex interactions

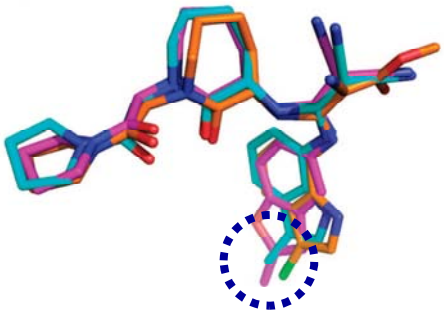
E.g. Cl-π interaction

- Cl-π interaction is an attractive interaction, where the major source of attraction is the dispersion force
- Calculated interaction energy is 2-3 kcal/mol depending on the chloro species
- Optimal distance is ca. 3.6 Å
- HF interaction is repulsive
- Electron correlation method, such as MP-2, needed to probe the interaction accurately
- For example – B.M.S. factor Xa inhibitor series[1]





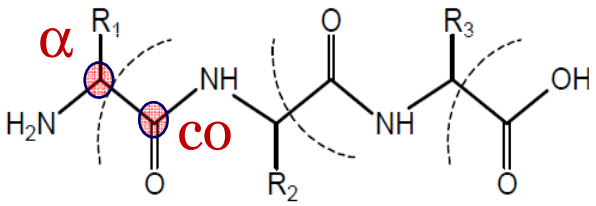
Imai *et al.*, *Protein Science*, *16*, 1229 (2008)



1. Shi, Sitkoff, et al., *J. Med. Chem.* **51**. 2008

Introduction to the FMO method

A wrapper for QM calculations in GAMESS



Fragmentation of peptide

$$E = \sum_{I}^{N} E_I + \sum_{I>J}^{N} \left(E_{IJ} - E_I - E_J \right)$$

PIE (Pair Interaction Energy)

Calculations for systems with 200-300 atoms are routinely run on a small 40-node cluster (~20/day)

- Full quantum computation of protein::ligand complexes - traditionally extremely large resources required for computing
- The fragment molecular orbital method (FMO) was proposed by **Kitaura** and co-workers (Kyoto)
  - Highly suitable for calculation of large (biological) systems in parallel computing environment
  - Implemented in GAMESS QM suite
  - PIEDA (Pair interaction energy decomposition analysis) provides detailed ligand/protein interaction information

Fukuzawa, K., *et al., Pure Appl. Chem.,* **75**(11-12), 2405-2410, 2003
Fedorov, D. G., and Kitaura, K., *J. Comput. Chem.*, **28**, 222-237, 2007

Scaling of standard QM (ab initio) and FMO

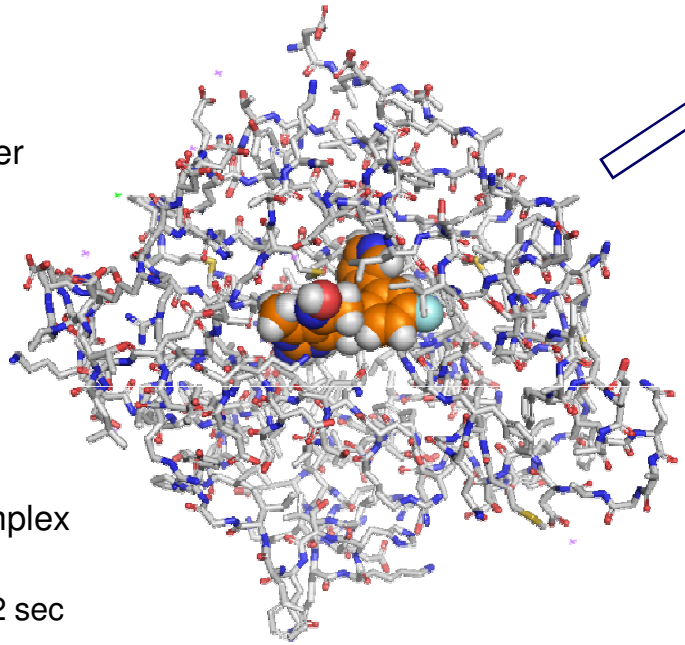
ab initio ~N^3: FMO ~N^2; N = number of atoms

Standard ab initio QM calculation has a scaling factor of ~N^3

water
N = 3 atoms

cpu time = **1 sec**

608 times larger



HSP90/inhibitor complex
N = 1823 atoms

cpu time = 224755712 sec

7.12 years

FMO calculation has a scaling factor of ~N^2

Calculation of the HSP90/inhibitor complex can be completed in;

4 days

Typical FMO calculations run at Evotec take

2-3 hours

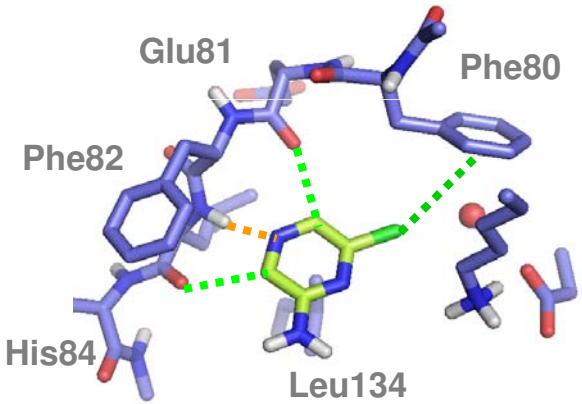
Fukuzawa, K., *et al., Pure Appl. Chem.,* **75**(11-12), 2405-2410, 2003
Fedorov, D. G., and Kitaura, K., *J. Comput. Chem.*, **28**, 222-237, 2007

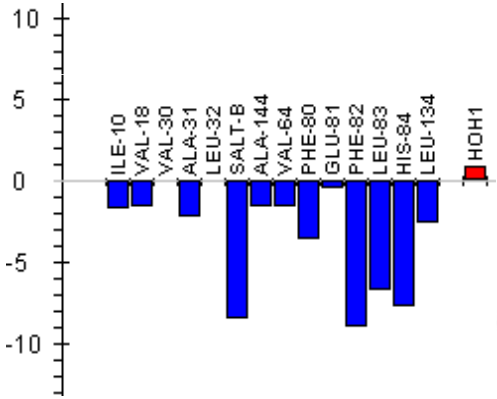
Application of FMO Calculations

Analysis & education

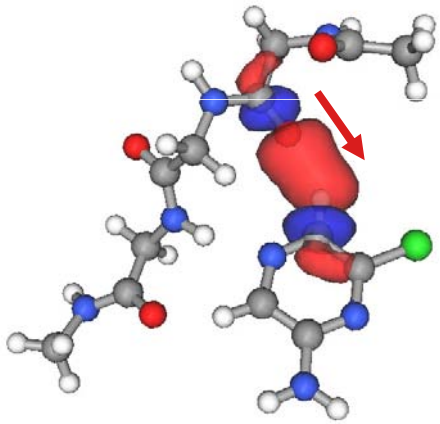
- Fragment Molecular Orbital (FMO) QM calculations can be used to assess the interaction enthalpy between a small molecule and each amino acid residue in the binding site of the protein
  - Analysis of Paired Interacting Orbitals (PIO) and by Pair Interaction Energy Decomposition Analysis (PIEDA) can give valuable insight into which are the key interactions

PDB: 1WCC
IC_{50} = 350 µM
-48.40 kcal/mol

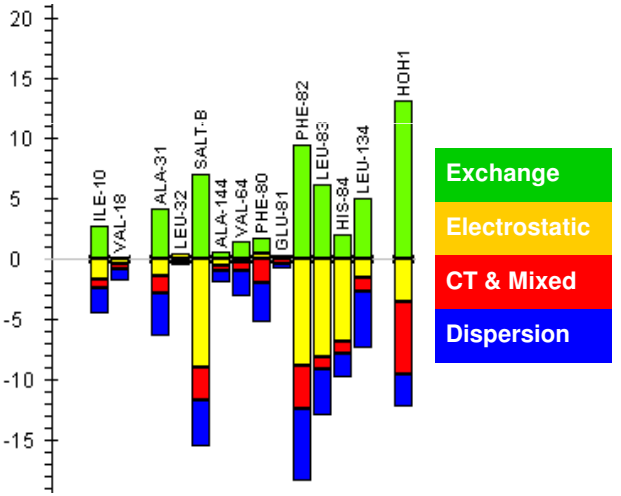




PIO analysis



PIEDA diagram

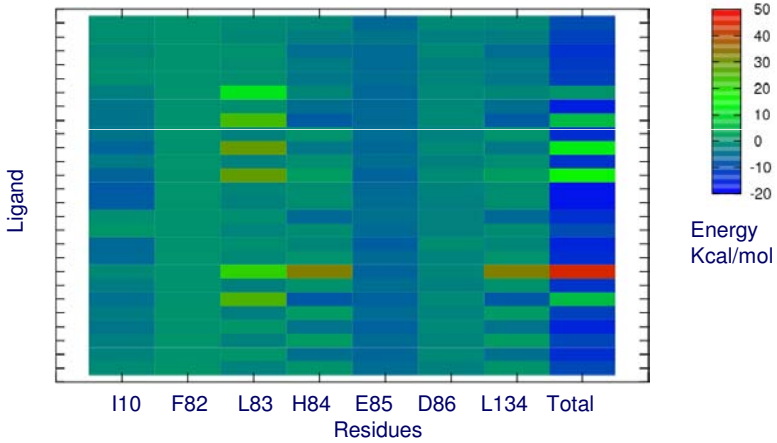


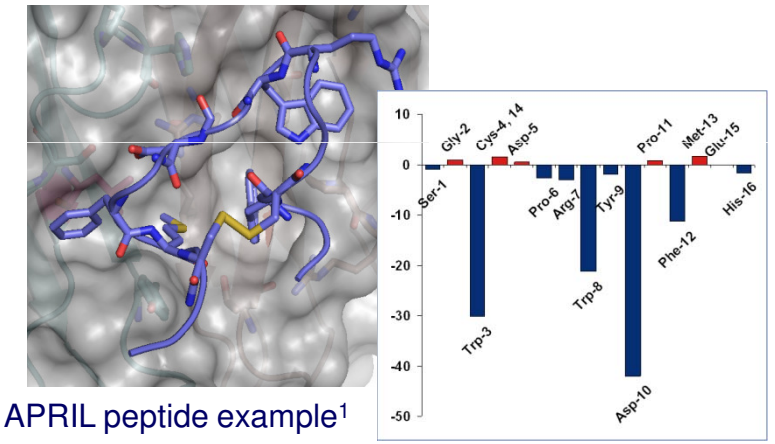
M.P. Mazanetz *et al.*, *J. Cheminform.*, 2011, **3**:2.

Application of FMO Calculations

Matrices of calculations and PPI target structures

- Fragment Molecular Orbital (FMO) QM calculations can be used to assess the interaction enthalpy between a small molecule and each amino acid residue in the binding site of the protein
  - Analysis of Paired Interacting Orbitals (PIO) and by Pair Interaction Energy Decomposition Analysis (PIEDA) can give valuable insight into which are the key interactions
  - FMO results may not correlate directly with activity data as solvation and entropy effects are not considered





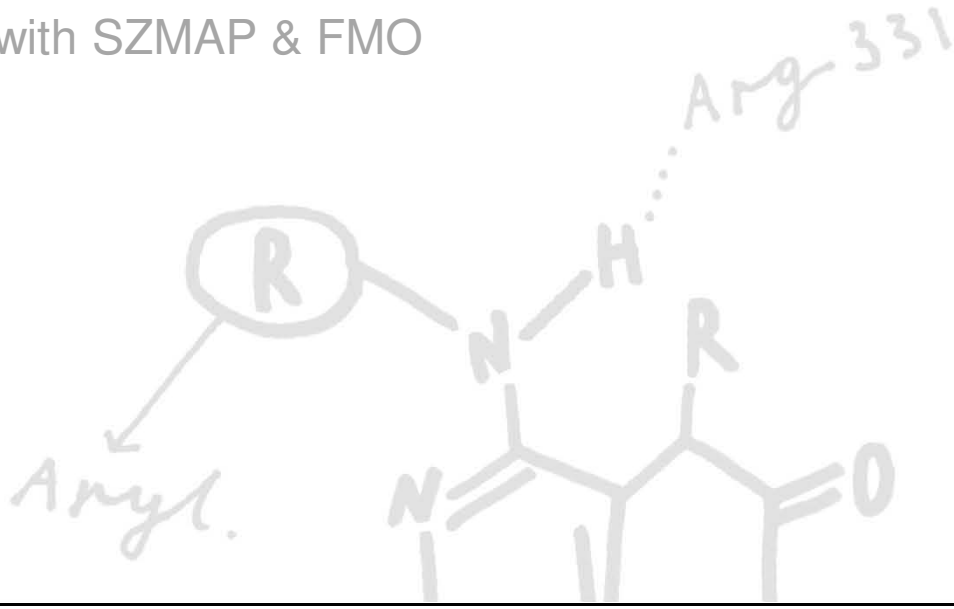
APRIL peptide example[1]

M.P. Mazanetz *et al., J. Cheminform.*, 2011, **3**:2.

evotec

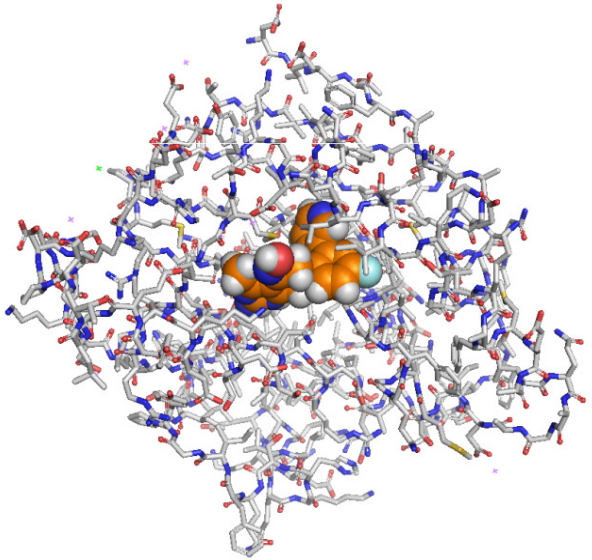
FMO for SBDD

- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with SZMAP & FMO

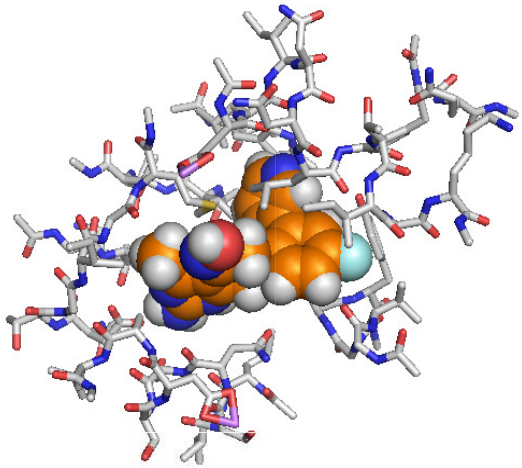


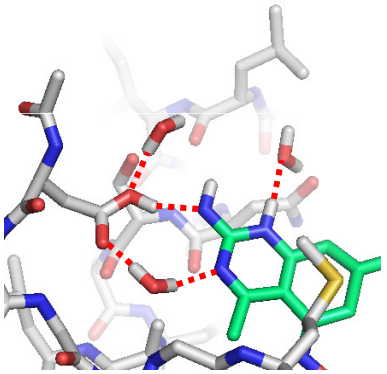
Preparation of the input: truncated system

Typical setup of the input structure and method



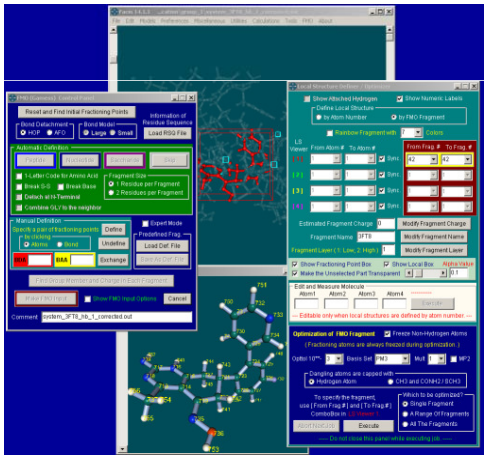
Residues within 6Å from the ligand are isolated, C-terminal N-methylated, N-terminal acetylated, some residues are removed/added depending on the local substructure





Hydrogens and H-bond network of waters (if any included) are generated/optimized using 'Protonate 3D' tool in MOE

For the ligand whose X-ray structure is not available, the molecule is modelled based on the template X-ray structure and its energy minimized within the binding site while the coordinates of key atoms are constrained



Input files are prepared using Facio† as a GUI to GAMESS QM suite‡

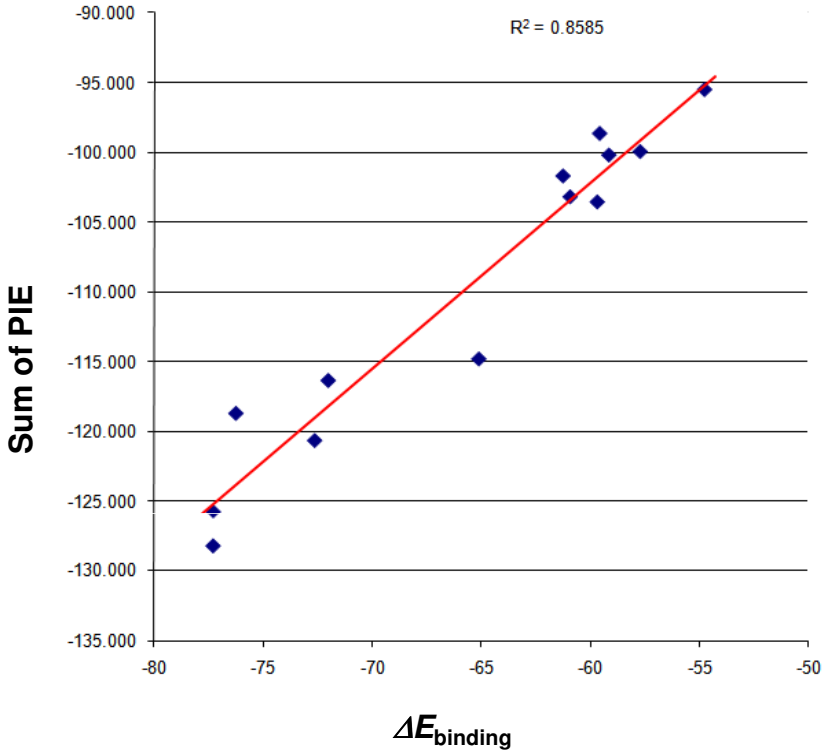
GAMESS FMO calculations are performed typically at MP2/6-31(+)G* theory level with PIEDA option

‡ M.W.Schmidt, K.K.Baldridge, J.A.Boatz, S.T.Elbert, M.S.Gordon, J.H.Jensen, S.Koseki, N.Matsunaga, K.A.Nguyen, S.Su, T.L.Windus, M.Dupuis, J.A.Montgomery *J. Comput. Chem., 14, 1347-1363(1993).*

† M. Suenaga, *J. Comput. Chem. Jpn., Vol. 4, No. 1 pp. 25-32 (2005)*
M. Suenaga, *J. Comput. Chem. Jpn., Vol. 7, No. 1 pp. 33-53 (2008)*

Correlation between Sum of PIE and $\Delta E_{binding}$

Using FMO as just a scoring function (from Hsp90 / ligand complex)



- Ligand binding is often calculated by the following scheme and requires three separate calculations

$$\Delta E_{binding} = E_{complex} - E_{apo} - E_{ligand}$$

- Sum of PIE for a ligand::protein complex (**single calculation**) is a good estimate for the binding energy
  - Linear relationship between Sum of PIE and $E_{binding}$
  - Contains some errors due to the ligand/protein polarisation
  - Other possible source of errors not accounted for by FMO calculation such as solvation energy and entropy terms has far larger impact on the results

Optimum cut-off for FMO-based energy estimation

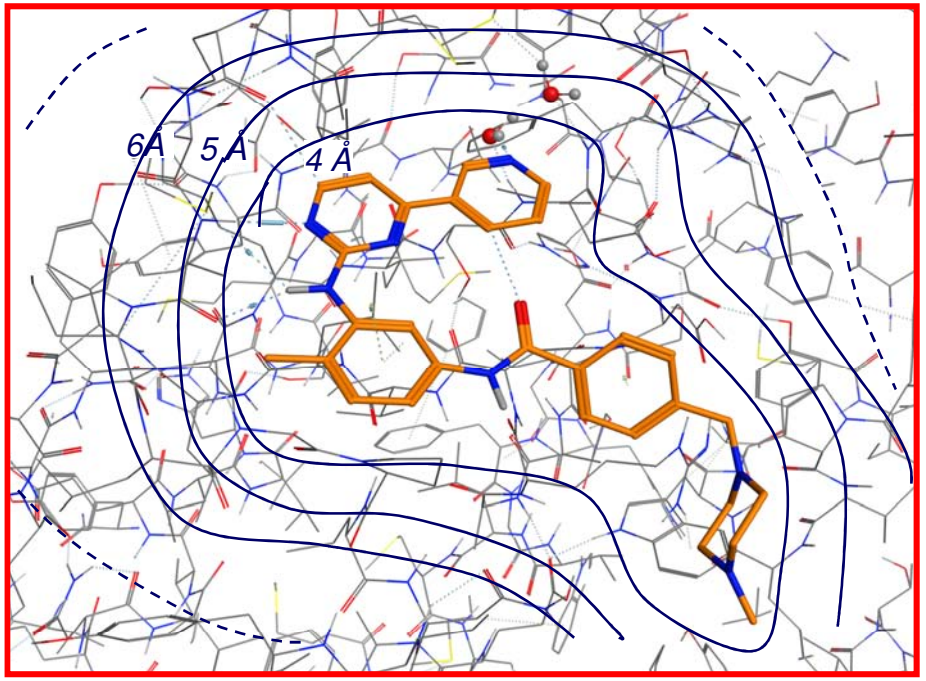
Gleevec bound to Syk

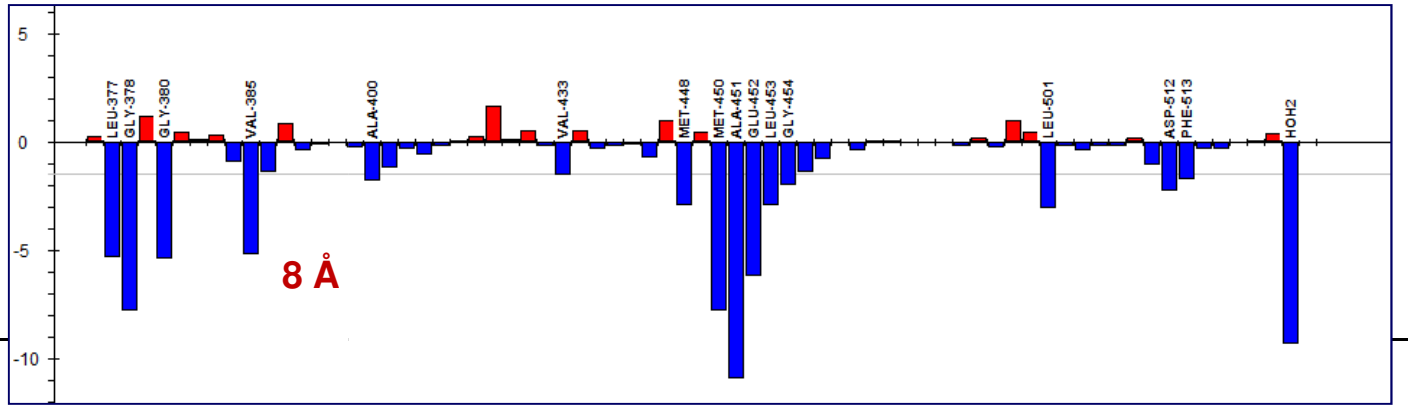
Gleevec bound to Syk

PDB id of the crystal structure used: 1XBB

Resolution = 1.57 Å

- Does the cut-off applied in FMO calculation affect the final estimated interaction energy between a ligand and its target?
- Interaction energy between Gleevec and Syk calculated for several cut-off distances: 4, 5, 6, 7, 8, 10, 90 *Å*



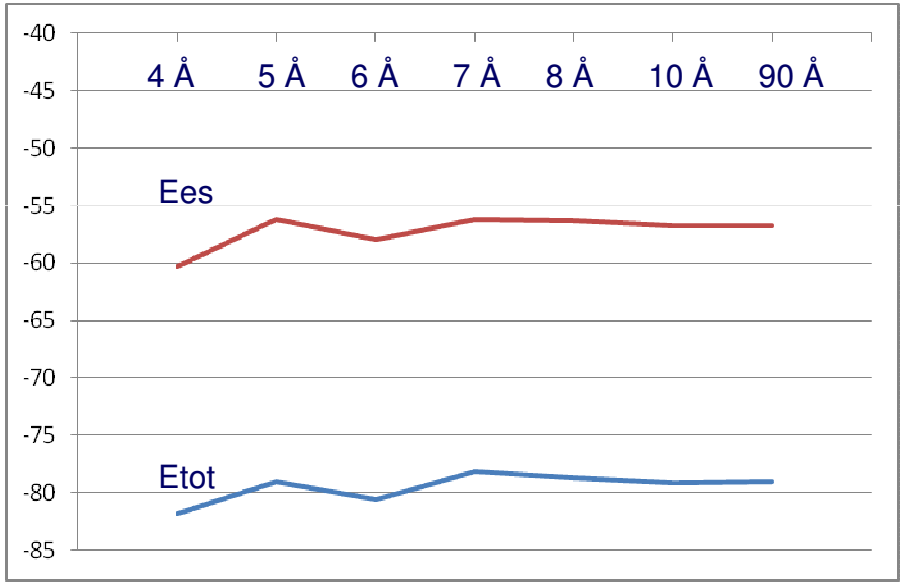


Optimum cut-off for FMO-based energy estimation

Total Ligand-Protein interaction energy plotted versus the cut-off used for the calculation

- Deviation in Etot are whithin 4% and mostly depend on differences in Ees
- The number of residues with Etot>1.5 kCal remains constant

	4 Å	5 Å	6 Å	7 Å	8 Å	10 Å	90 Å
Etot	**-81.82**	**-79.07**	**-80.54**	**-78.19**	**-78.75**	**-79.16**	**-78.99**
Ees	-60.36	-56.22	-57.91	-56.13	-56.33	-56.76	-56.71
Eex	63.99	62.71	62.62	62.79	62.81	62.88	63.10
Ect_mix	-22.08	-21.37	-21.90	-22.10	-22.11	-22.17	-22.23
Edisp	-63.39	-64.18	-63.35	-62.75	-63.12	-63.10	-63.15
# resid.	31	41	54	60	71	95	270
# resid. E > 1.5	17	17	17	16	16	16	16

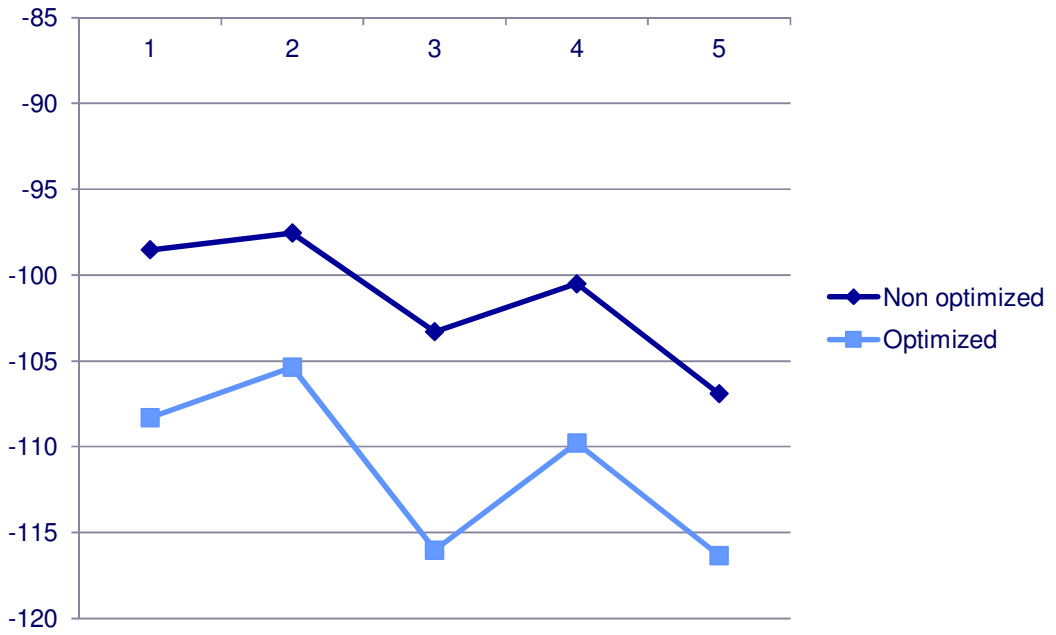


How much difference MM optimization make?

Very little

- Ligand optimized using MMFF; protein kept fixed
- FMO PIEDA calculation performed before and after optimization
- The energy trend is mostly conserved after the minimization
- Same trend seen in series where FMO correlated with binding E and cases where it didn't
- Does QM/MM change things?

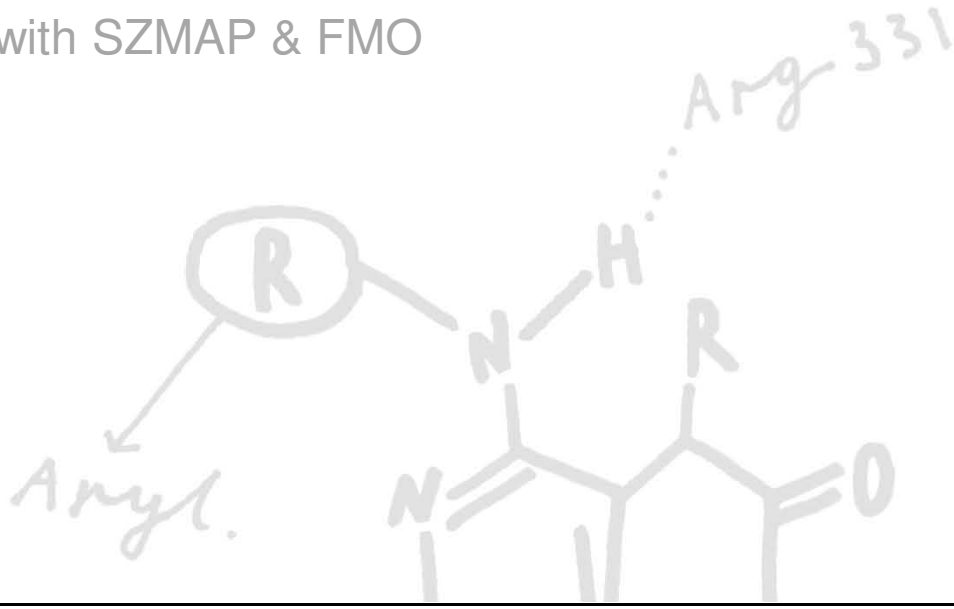
	Etot Non optimized	Etot Optimized
3571	-98.526	-108.309
BIM_3571_JH-NH2	-97.548	-105.359
BIM_3571_JH-CF3	-103.301	-116.031
BIM_3571_JH-NH2bis	-100.514	-109.779
BIM_3571_JH-CF3bis	-106.904	-116.347



evotec

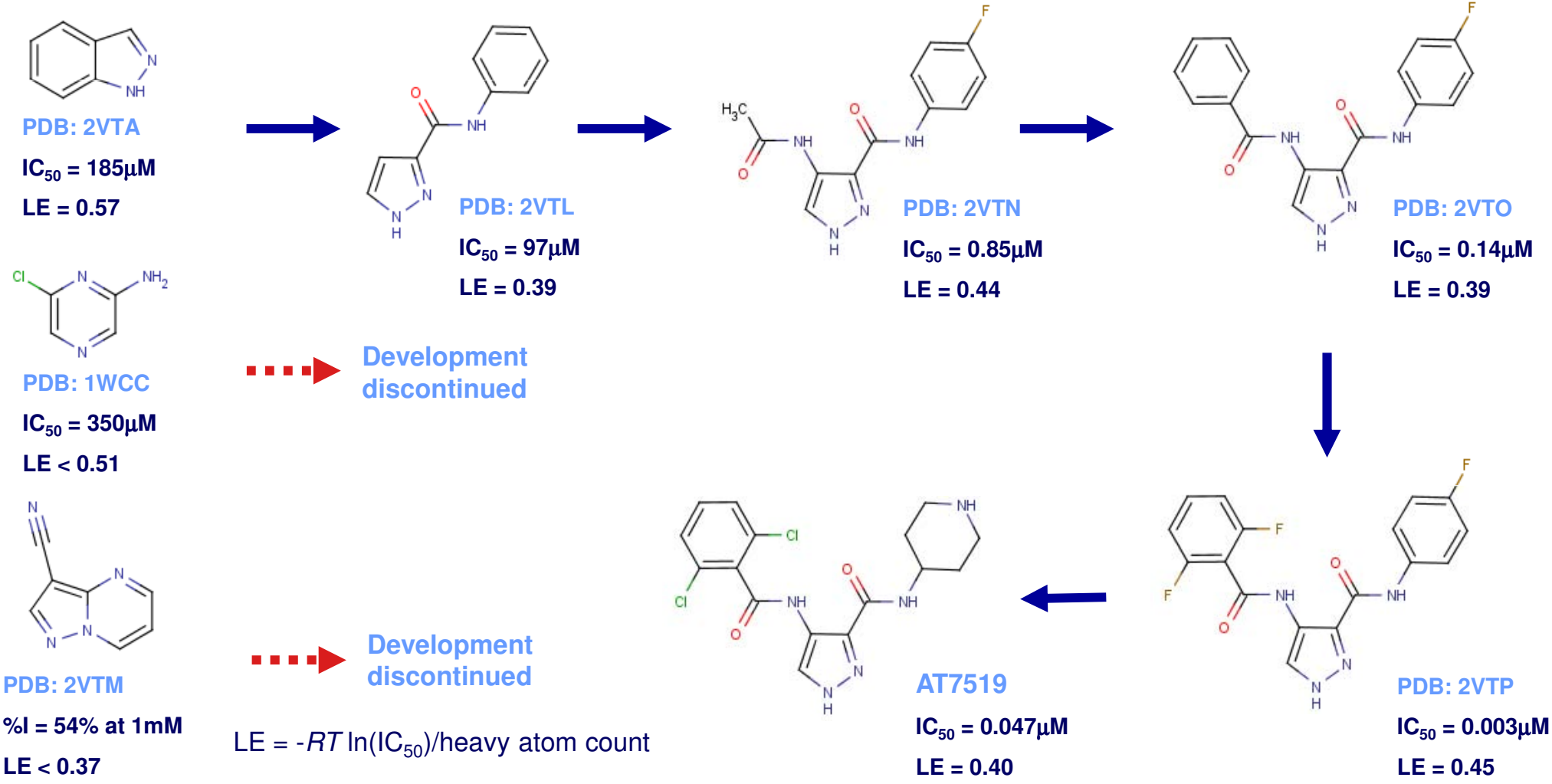
FMO for SBDD

- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with SZMAP & FMO



Application of FMO to FBDD

Astex AT7519 (CDK2 inhibitor) as an example



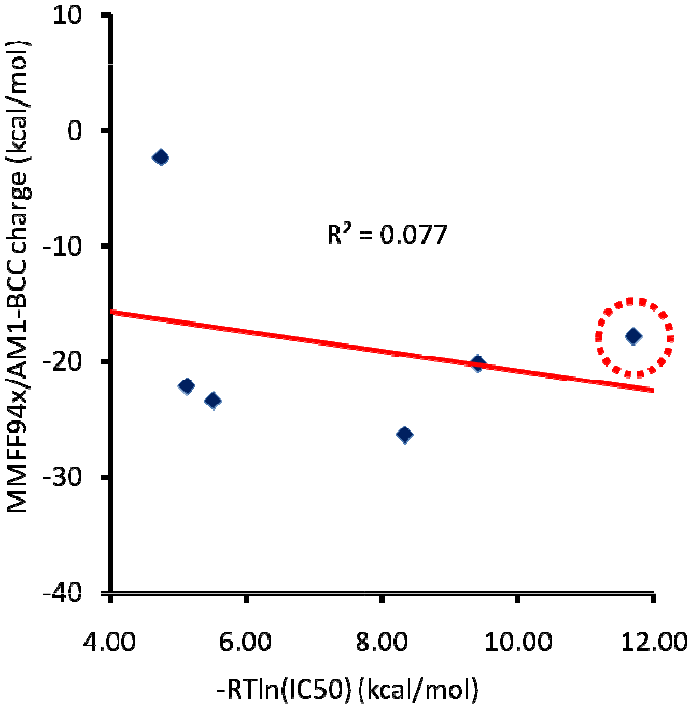
P. G. Wyatt *et al.*, *J. Med. Chem.* **2008**, *51*, 4986-4999

M. Congreve *et al.*, *J. Med. Chem.* **2008**, *51*, 3661-3680

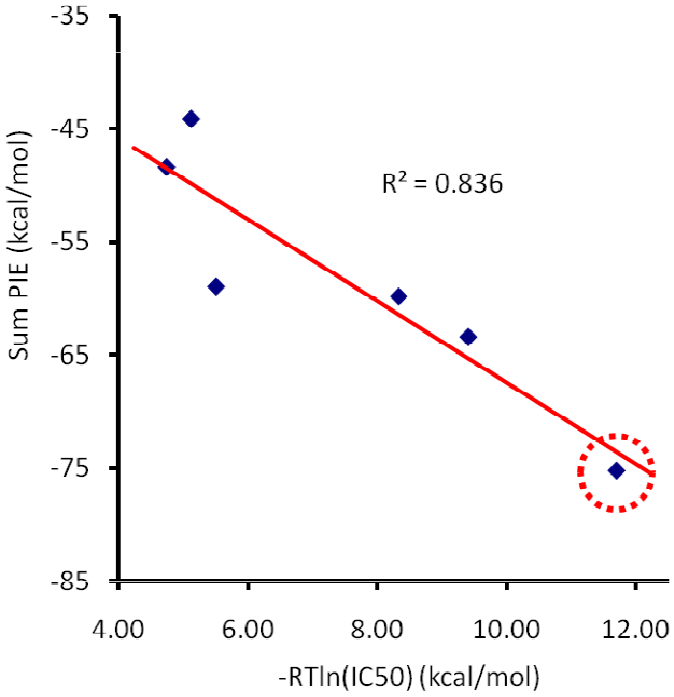
Application of FMO to FBDD

Sum PIE/pIC50 and MM/AM1-BCC binding energy correlation

- AM1-BCC charge emulates ab initio HF 6-31G* ESP charge and considered to be superior to static MM charges
  - MM binding energy was calculated using MMFF94x charge on protein, AM1-BCC charge on ligand



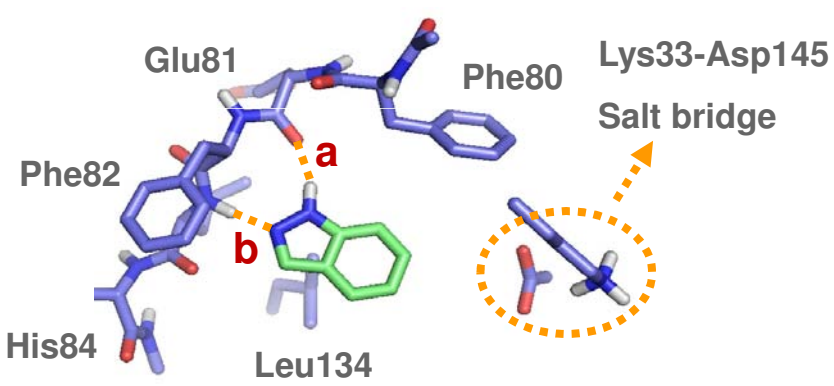
Binding energy calculated using MMFF94x with AM1-BCC charge on the ligand

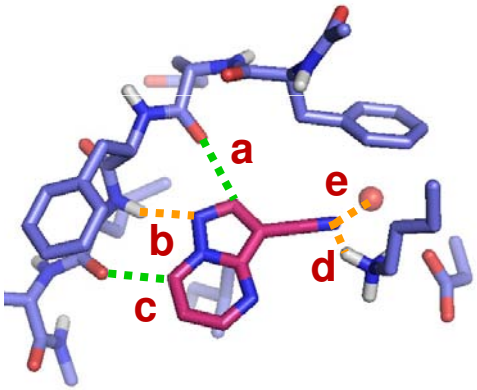


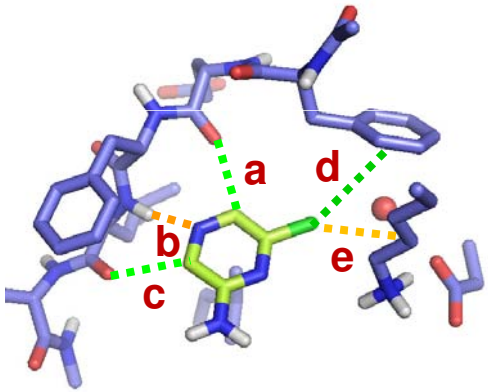
FMO Sum PIE

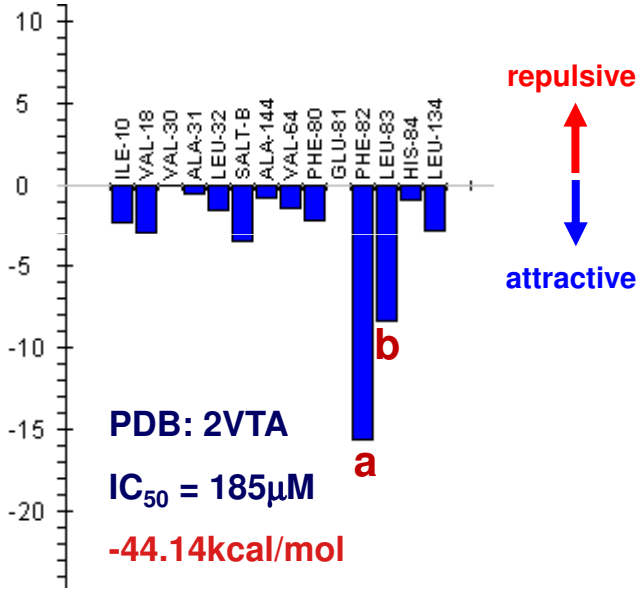
Application of FMO to FBDD

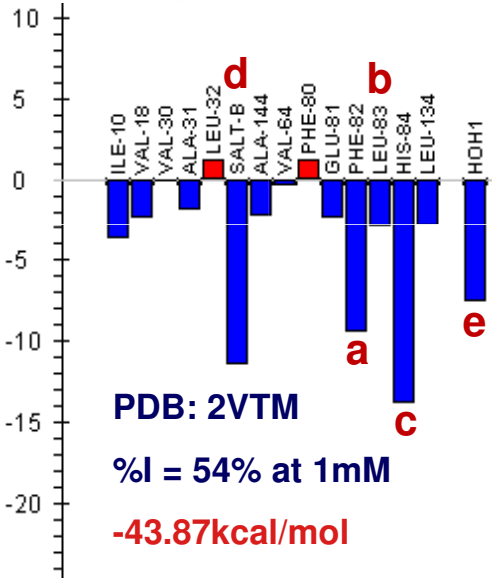
FMO analysis of fragment/protein interaction: PIE

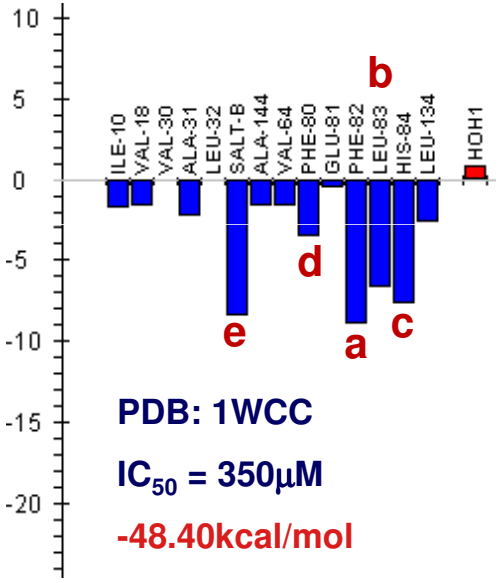












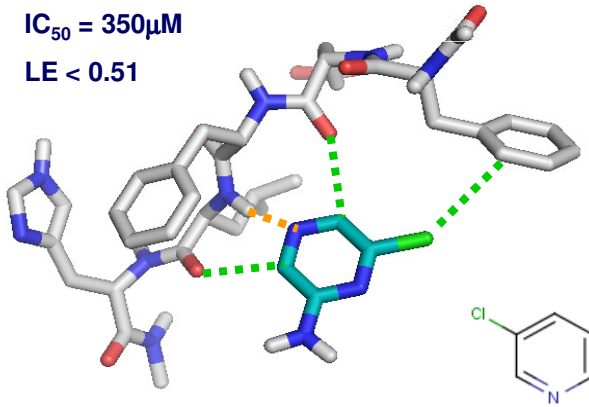
QM Virtual SAR expansion using FMO

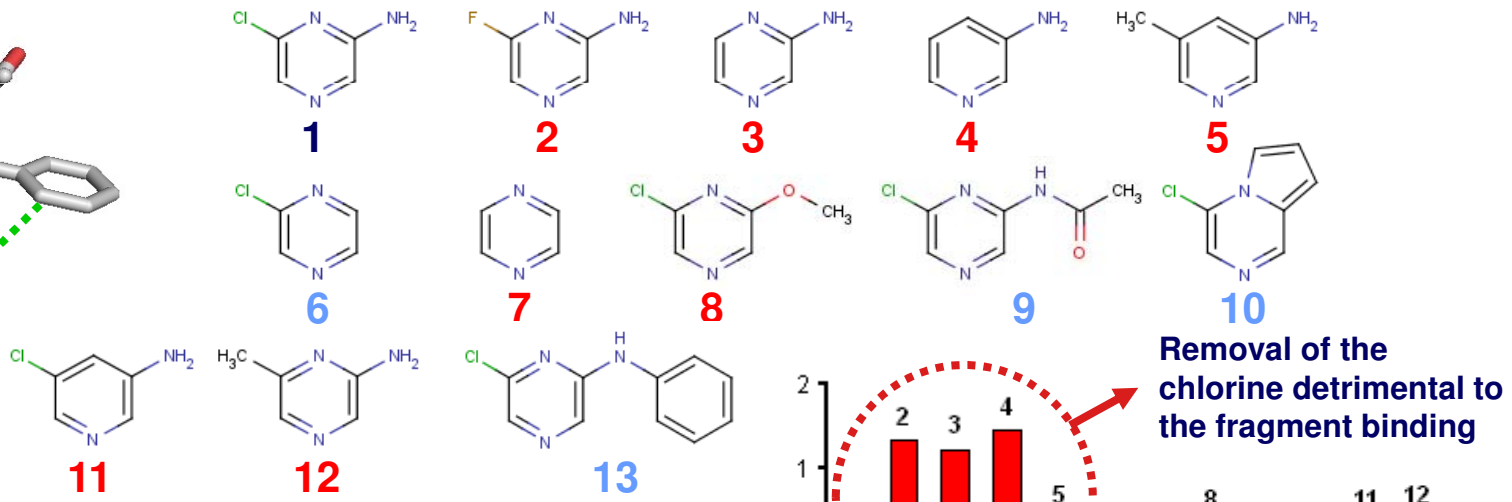
1WCC (CDK2) core modifications: Prediction

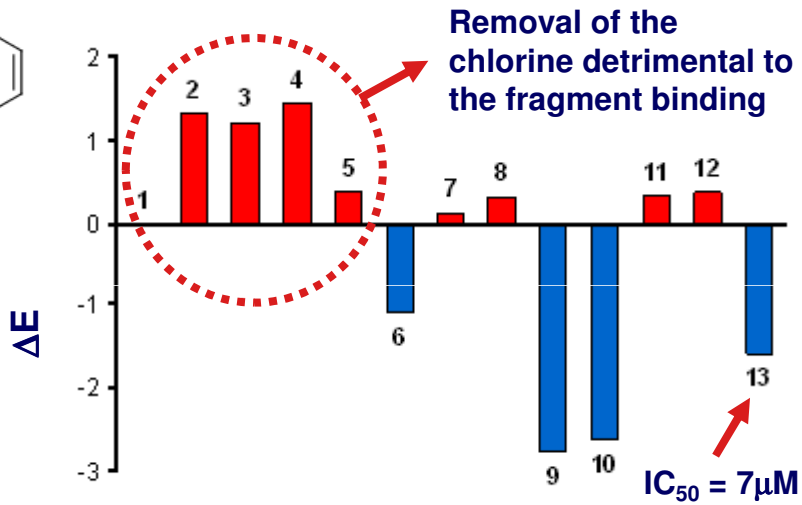
PDB: 1WCC

IC_{50} = 350µM

LE < 0.51







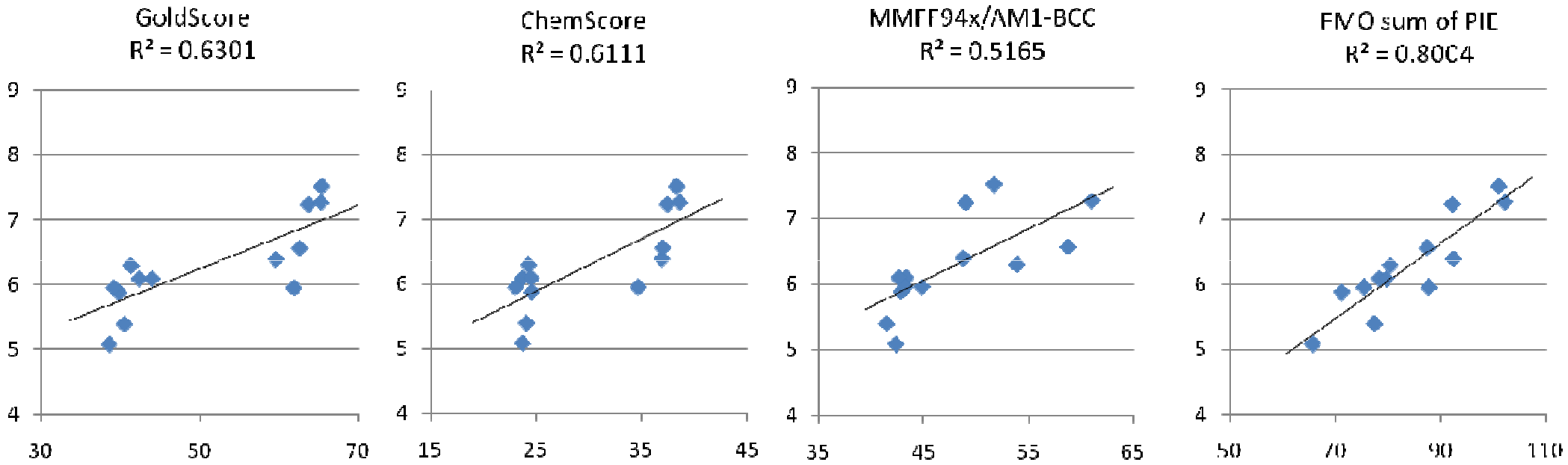
- Medium throughput FMO analysis can be rapidly carried out to answer SAR questions
- The technique is highly effective for prioritizing the initial fragment expansion directions or optimization for larger ligands

ΔE = Sum PIE – Sum PIE (1WCC fragment)

Comparing scoring function to docking scores

Including comparison to AM1-BCC charge set

- Data from a set of Hsp90 compounds from a congeneric series
- Calculations run from single crystal structure with small minimized changes

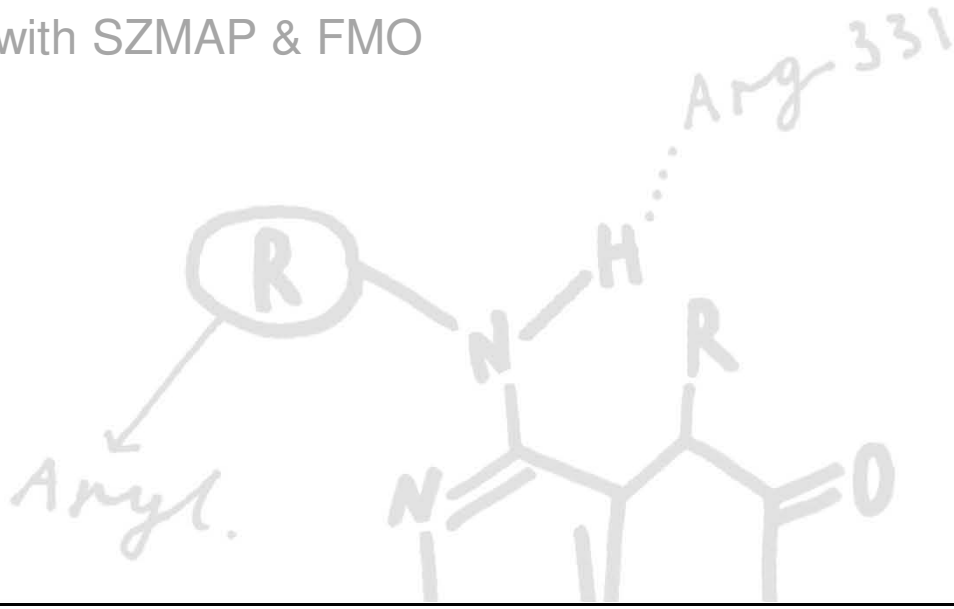


Ichihara et al. (2012). (Paper submitted).

evotec

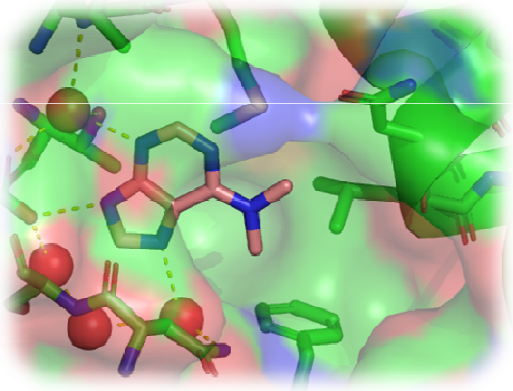
FMO for SBDD

- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with SZMAP & FMO

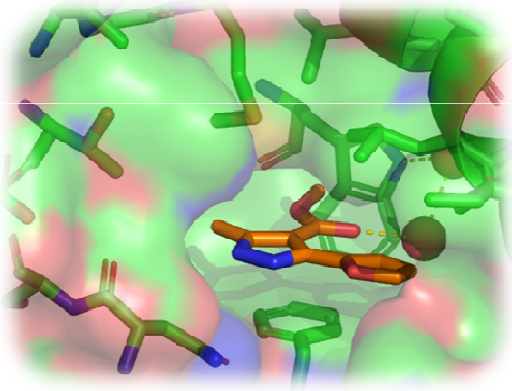


FMO analysis of Hsp90 fragment linking

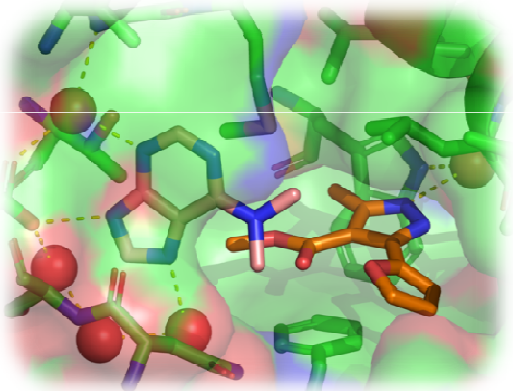
Do the two fragment binding energies really add up?



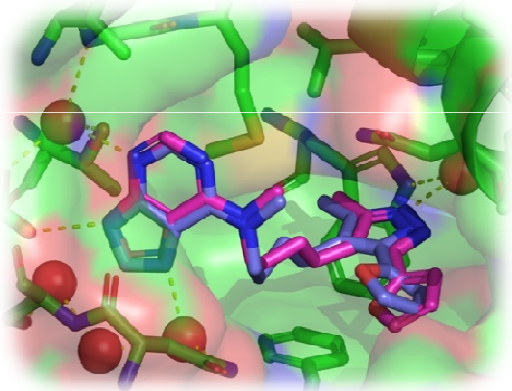
X-ray structure of A in ATP site



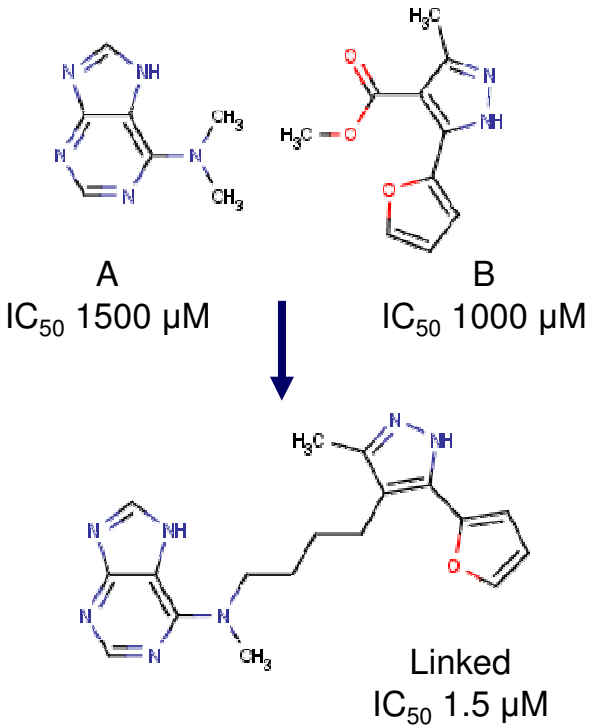
X-ray structure of B in Helical pocket



X-ray structure of dual complex



X-ray structure and *in-silico* predicted linked fragments, rmsd 0.49Å



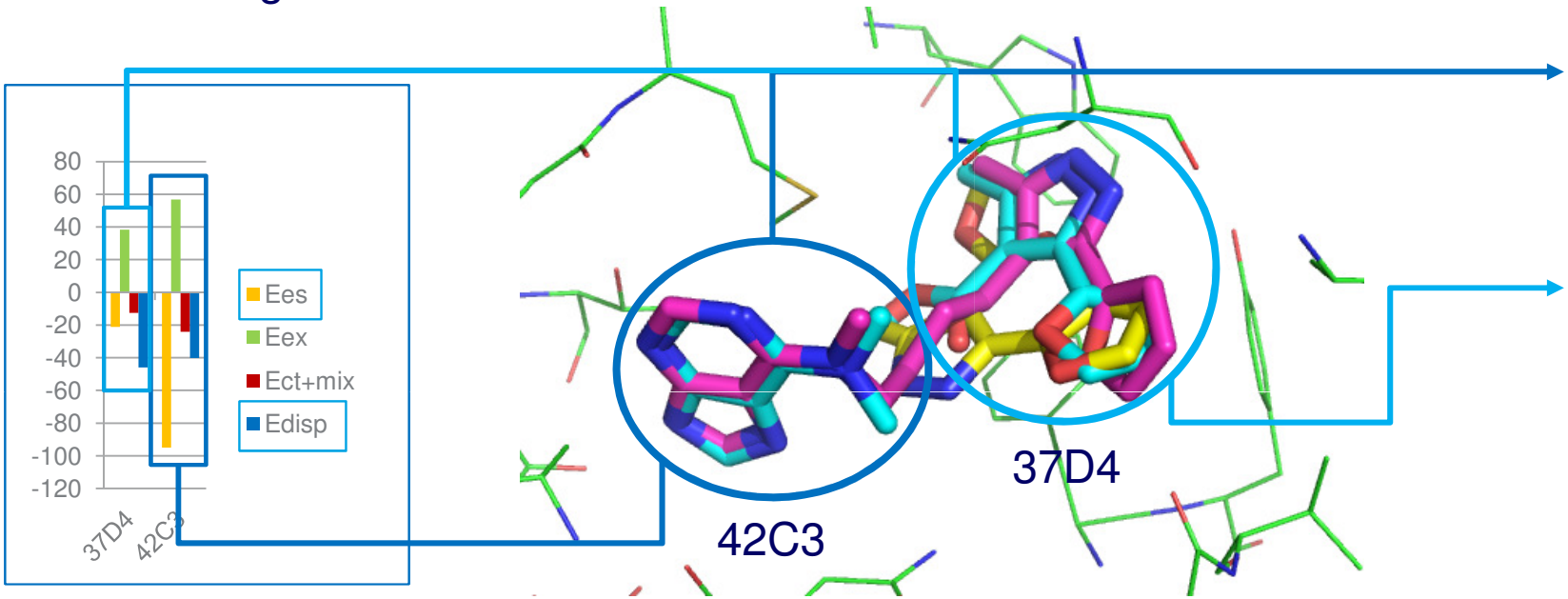
- Two fragment hits were linked based on the X-ray structure of the dual complex
- 1000 fold increase in potency achieved

[1]Barker *et al.* (2010). Discovery of a Novel Hsp90 Inhibitor by Fragment Linking. *ChemMedChem* 5(10):1697-700.

Use of FMO analysis to select fragments

Which to expand on, which to link to? Hsp90 example

- FMO can be used to select/prioritize fragments for expansion or linking
- Ratio of electrostatic and dispersive interactions predicts which fragments are good to expand on, and which a good to link to



- Good elec/disp. ratio
- Good for expansion – low RMSD

- Relatively highly dispersive
- Not good for expansion
- Good for linking to from other good ratio fragment

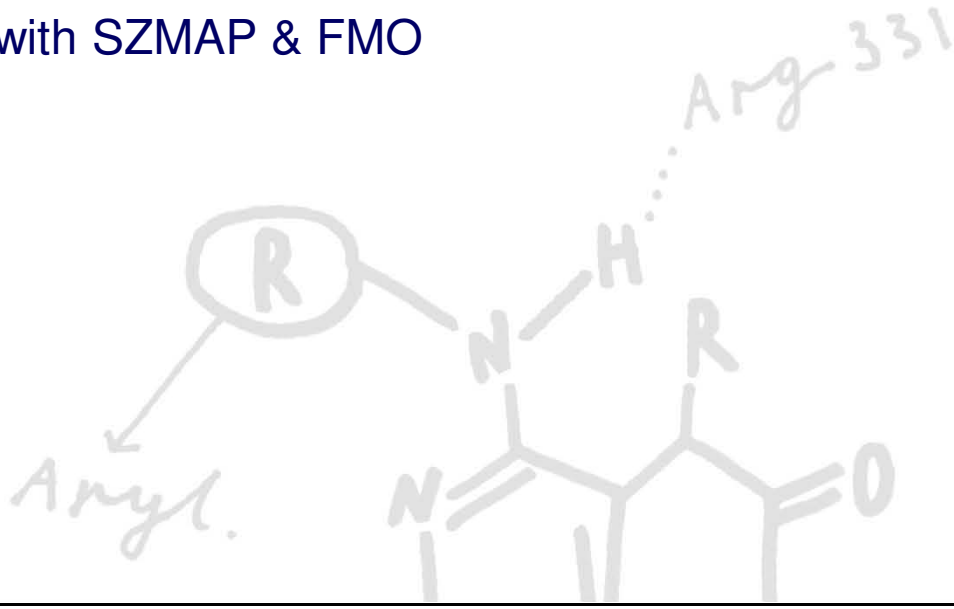
- Maintaining the electrostatic/dispersive balance in med.chem. is important for maintaining potency
  (too high elec – high desolvation penalty)

Ichihara et al. (2011). Compound Design by Fragment Linking. *Molecular Informatics. 30 (4) 298-306.*

evotec

FMO for SBDD

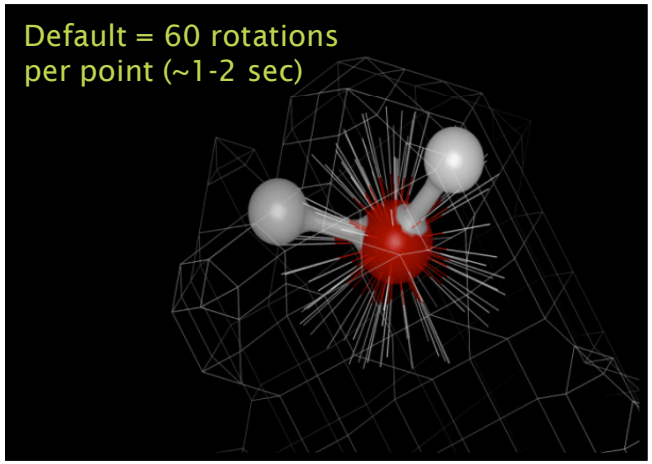
- Intro to the Fragment Molecular Orbital Method
- Testing a few FMO calc. set-up variables
- Some results – CDK2
- Fragment linking and selection – Hsp90
- Water Probe scoring with SZMAP & FMO

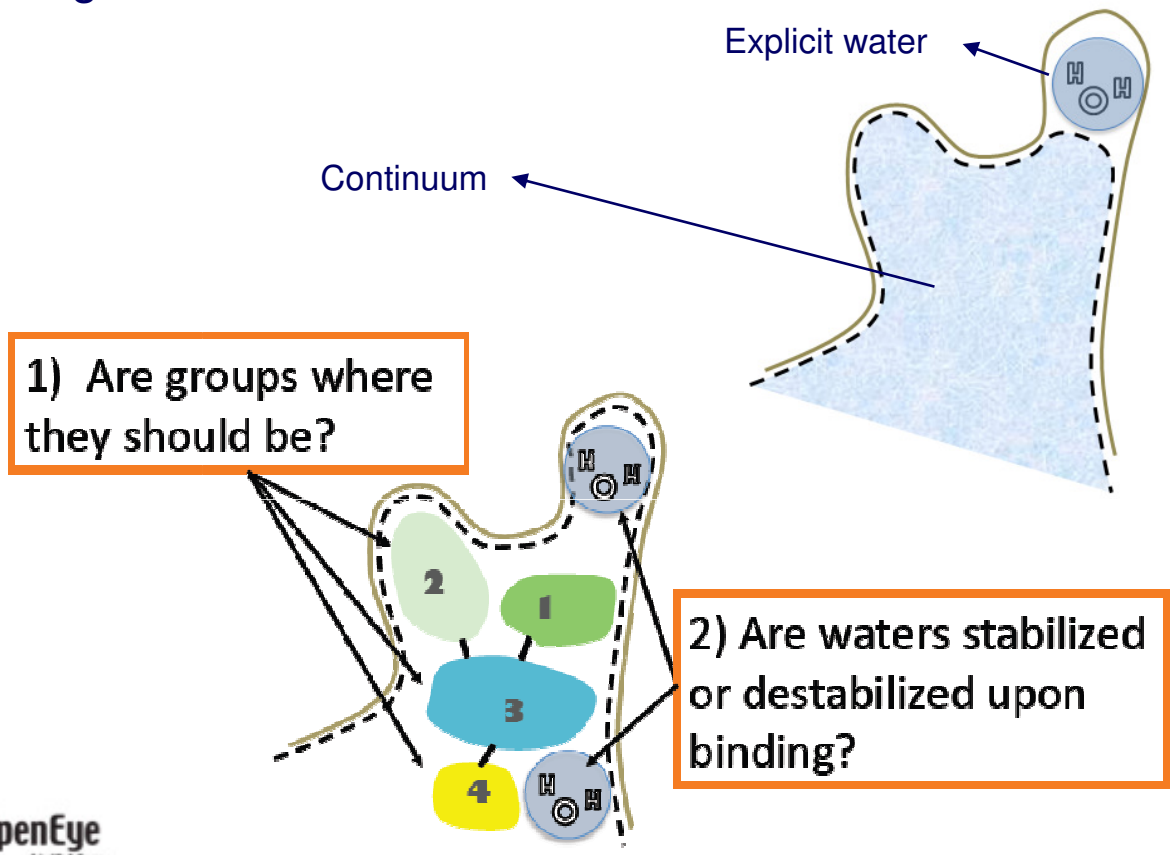


SZMAP

Semi-continuum theory

- It uses a single explicit probe water in a high-dielectric continuum solvent to rapidly map the magnitude and distribution of solvent energies near a molecular surface.
- How does it work?
  1. Place atomic water / neutral water
  2. Treat rest of water as continuum
  3. Sample orientations
  4. Repeat





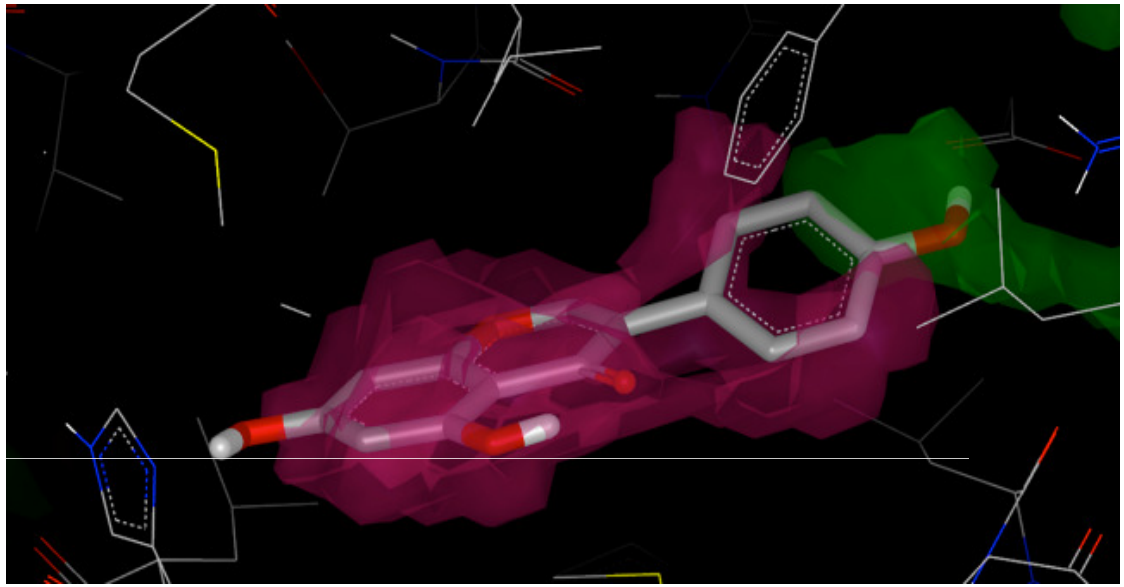
OpenEye Scientific Software

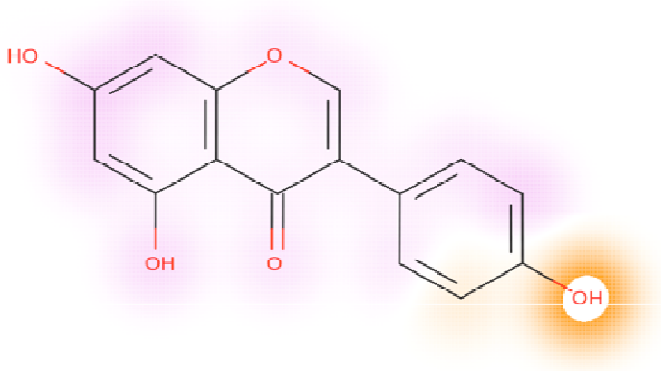
Thank you to Ant Nicholls & Mike Word from OpenEye

SZMAP & Grapheme

Displaying water chemical potential

- ER-β and Genistein (1QKM)

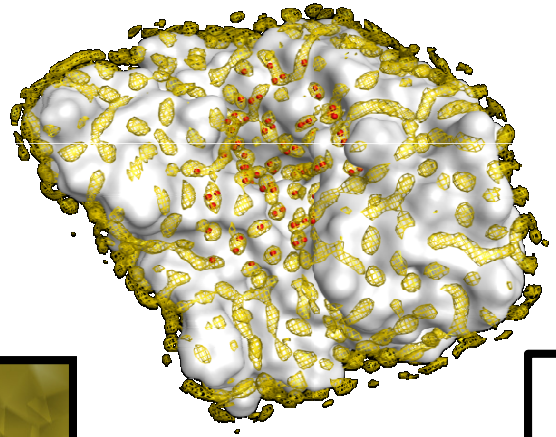




Assessing waters to guide chemistry design

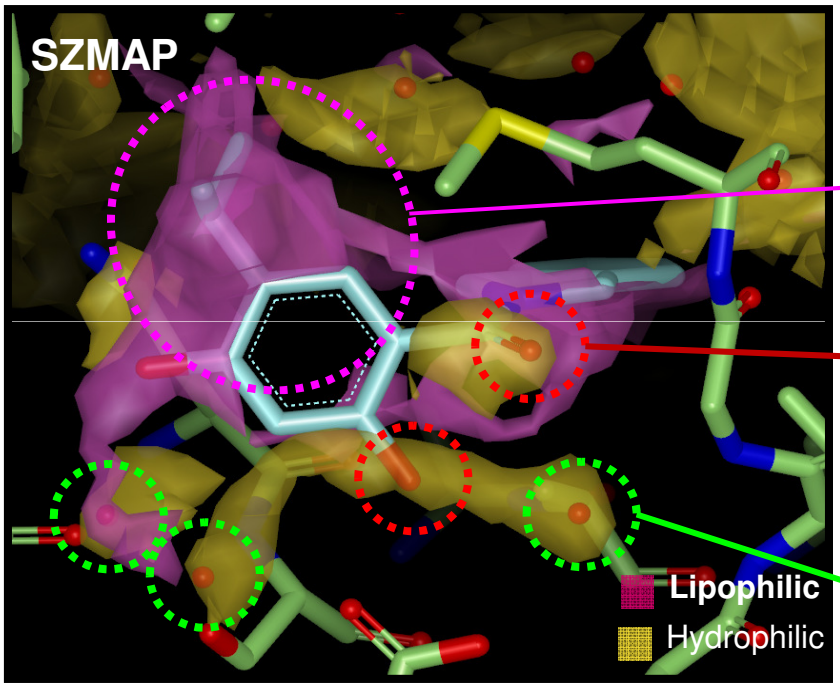
Mapping and scoring of water positions

- Crystal waters, molecular dynamics or semi-continuum simulations are used to predict water cluster positions



- Different analyses can then be used to score those water positions in terms of potential and energetics

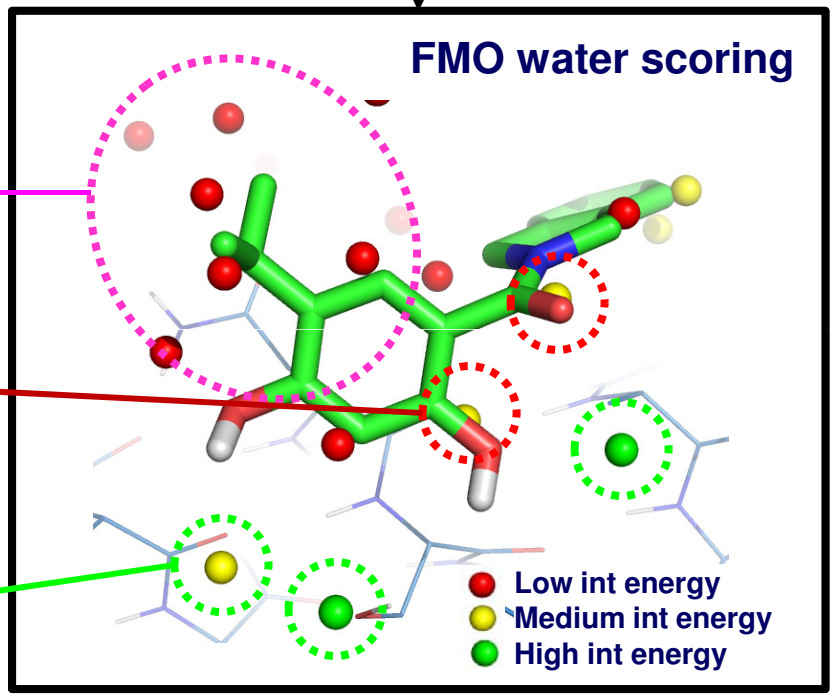
OpenEye



Replace low-scoring water (or lack of water) with hydrophobic motif

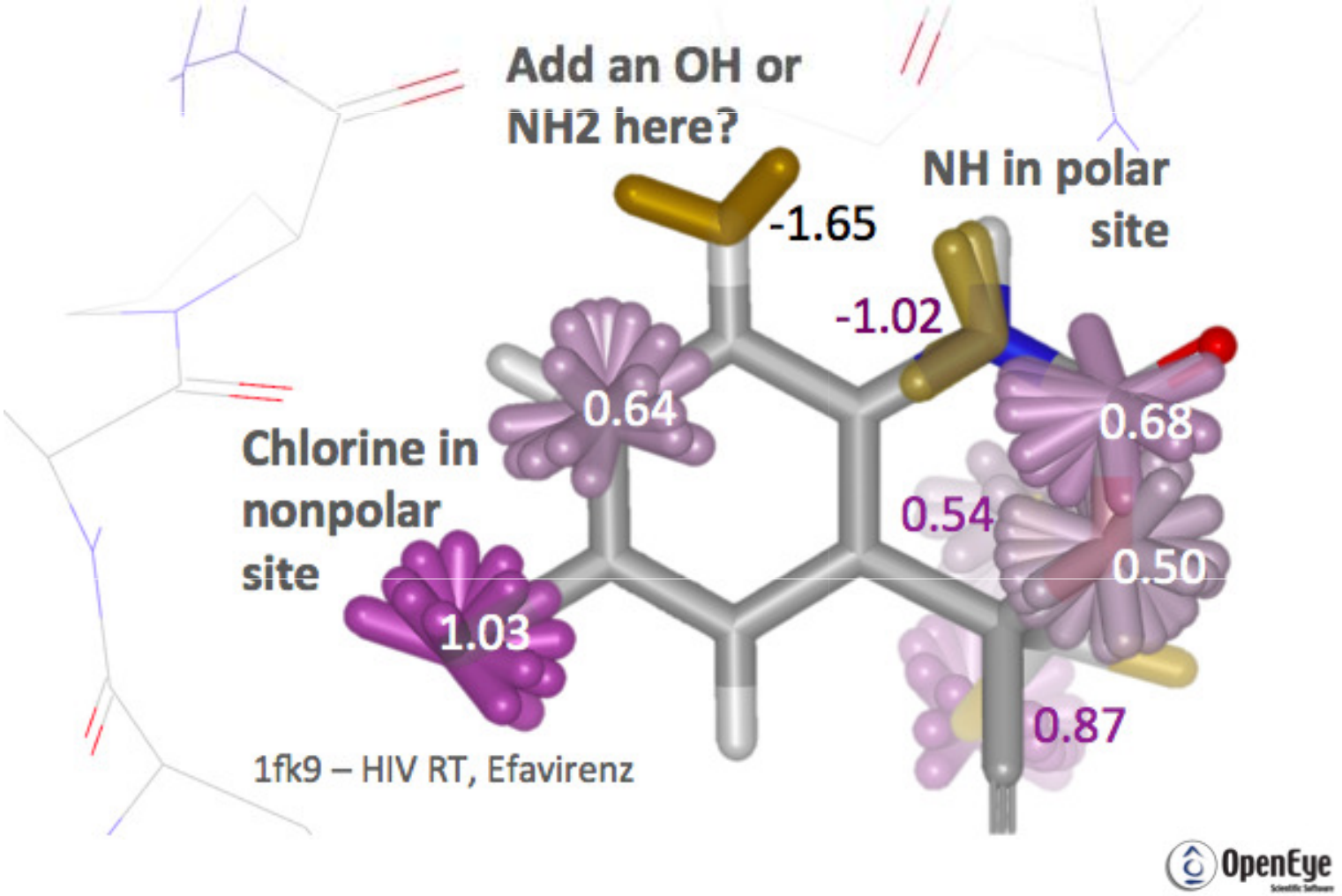
Replacement of medium water with H-bonding motif

Ordered waters can be addressed by H-bonding motifs



† A J Woodhead *et al., J. Med. Chem., 2010, 53, 5956*

Szmap or MD waters – correlation to water order

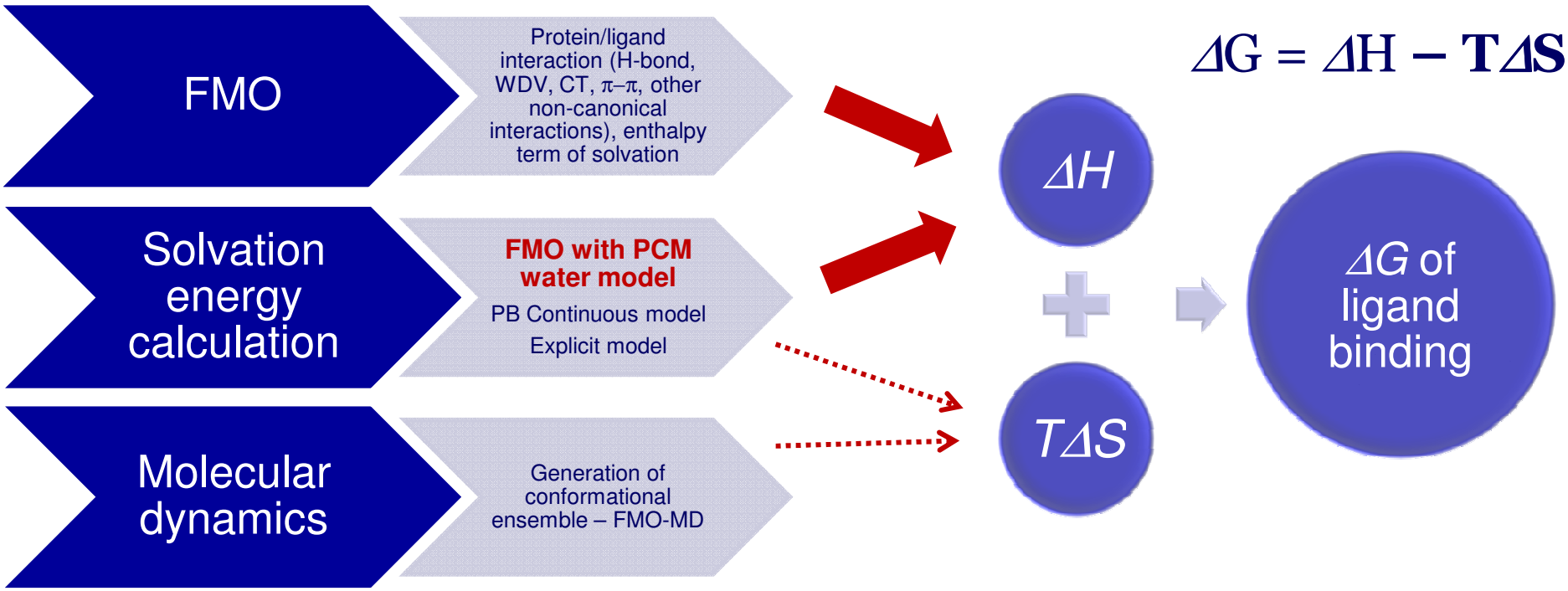


FMO for SBDD

- And one last thought . . .

Free energy of ligand binding and FMO energies

When enthalpy alone will not do!



Mazanetz et al. (2011). Prediction of cyclin-dependent kinase 2 inhibitor potency using the fragment molecular orbital method. J. Cheminformatics 3:2

evotec

Conclusions

And some words of caution!

- FMO enables QM calculations to be performed and results displayed in a way that can drive F/SBDD in a predictive and educational way
- FMO is an enthalpy calculation, so it works best within a single chemical series for which other non-enthalpy factors tend to cancel out
- If FMO results do not correlate with activity data, it is likely that other factors not included in the FMO calculations are dominant
  - Unexpected change in binding mode → does docking/xstal structures suggest this?
  - Protein conformational change → low mode MD show this?
  - Solvation effect (including active site water molecules) → does MM-PBSA or water analysis help?
  - Physicochemical property issues → make a graph!

Acknowledgments

Computational Chemistry Group

Osamu Ichihara (Schrodinger, Japan)

Michelle Southey

Michael Mazanetz

Alexander Heifetz

Inaki Morao

Mirco Meniconi

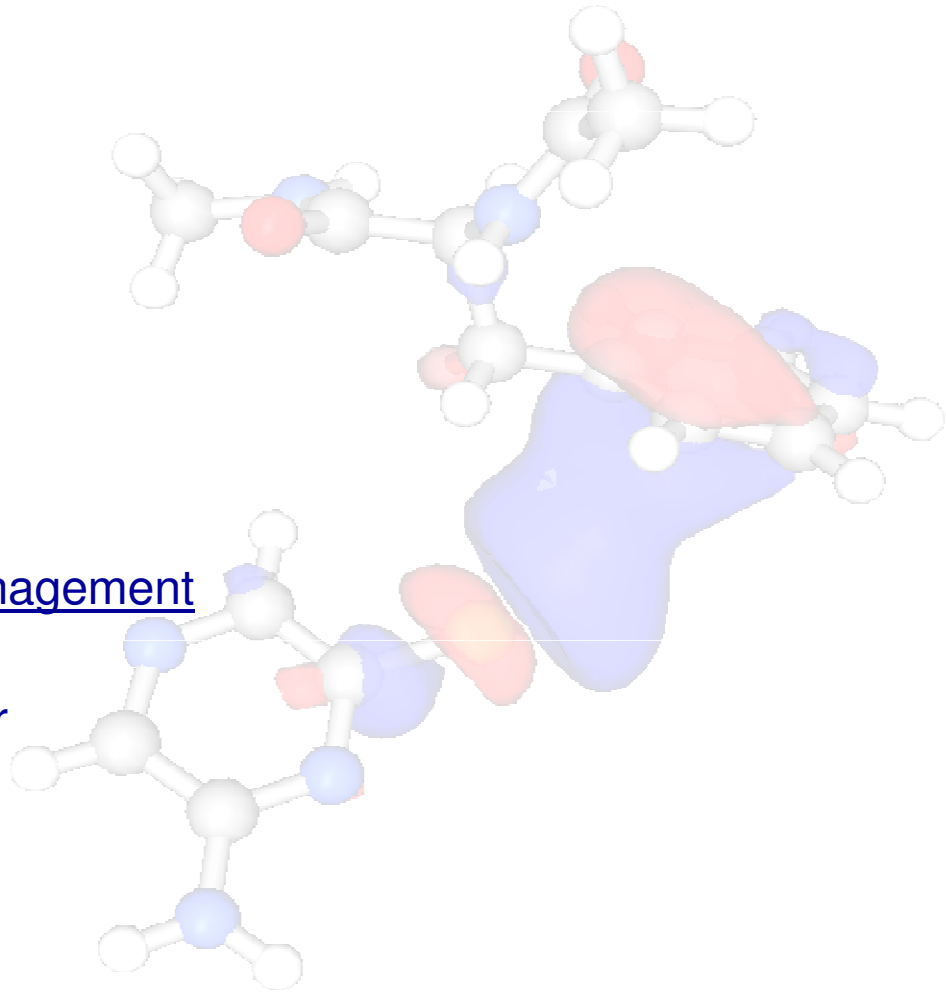
Daniel Warner

Tim James

Chemistry Management

Mark Whittaker

David Hallett



evotec

‘RESEARCH NEVER STOPS’

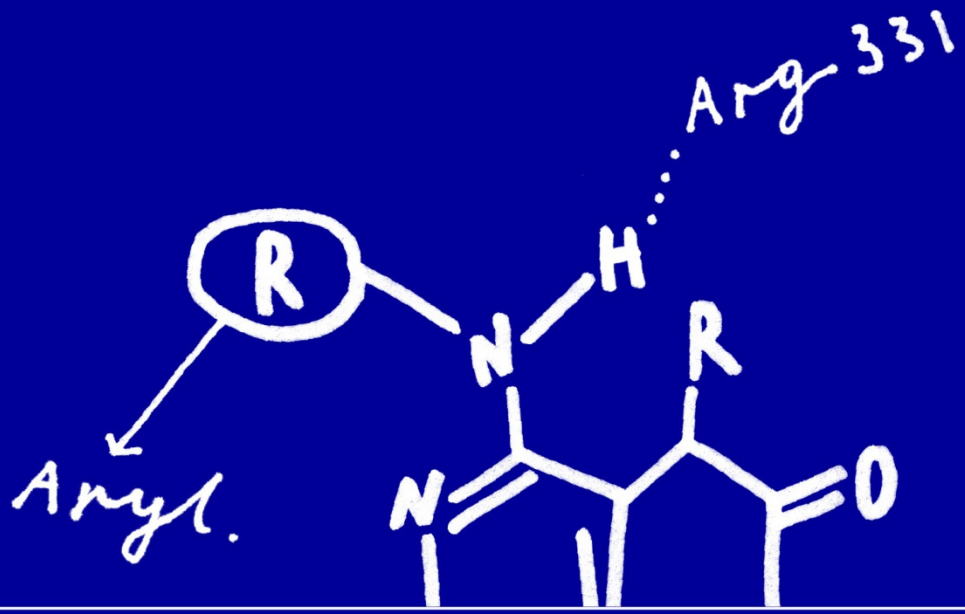
Building innovative drug discovery alliances

Your contact:

Dr. Richard J. Law

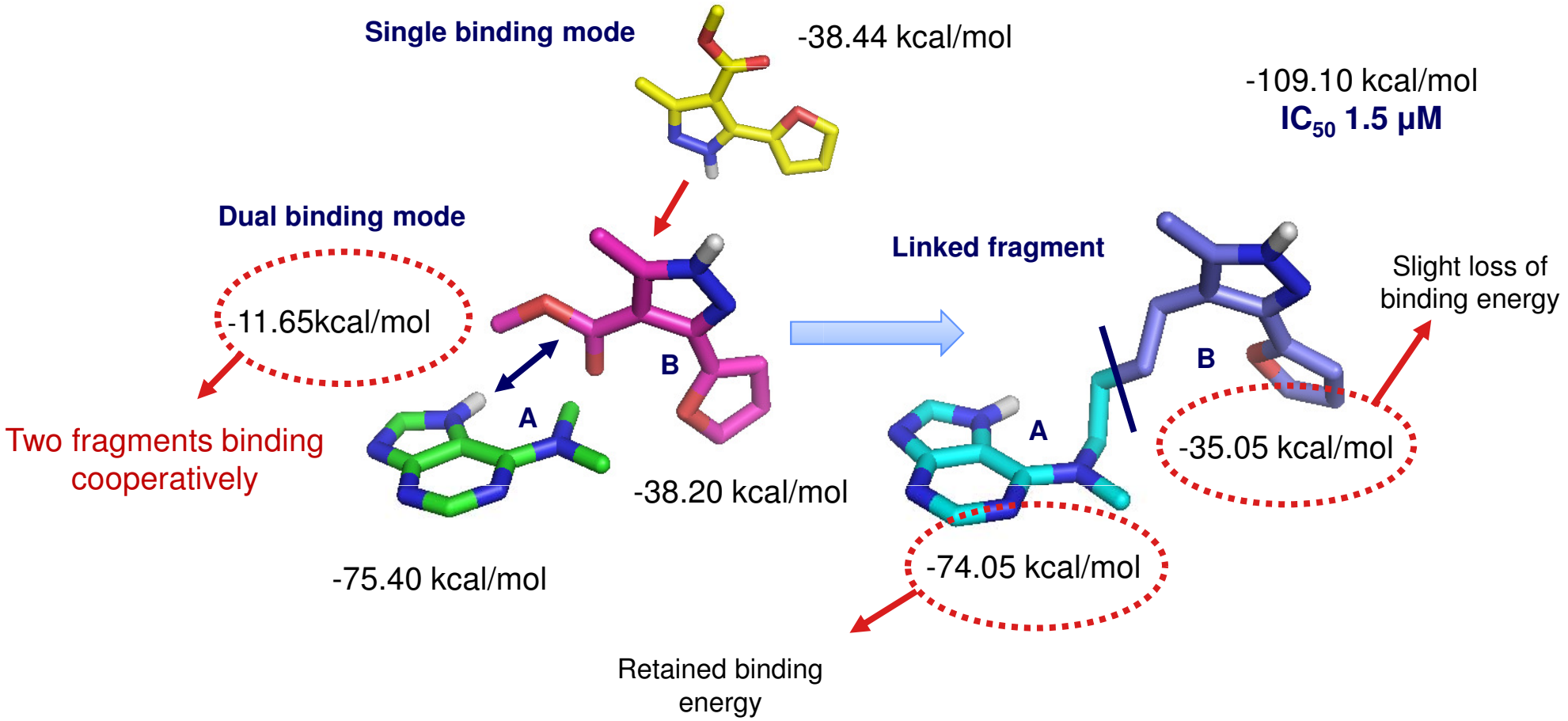
Group Leader, Computational Chemistry

+44 (0) 1235 861561
Richard.Law@evotec.com



FMO analysis of Hsp90 fragment linking

FMO binding energy analysis: dual and linked fragments

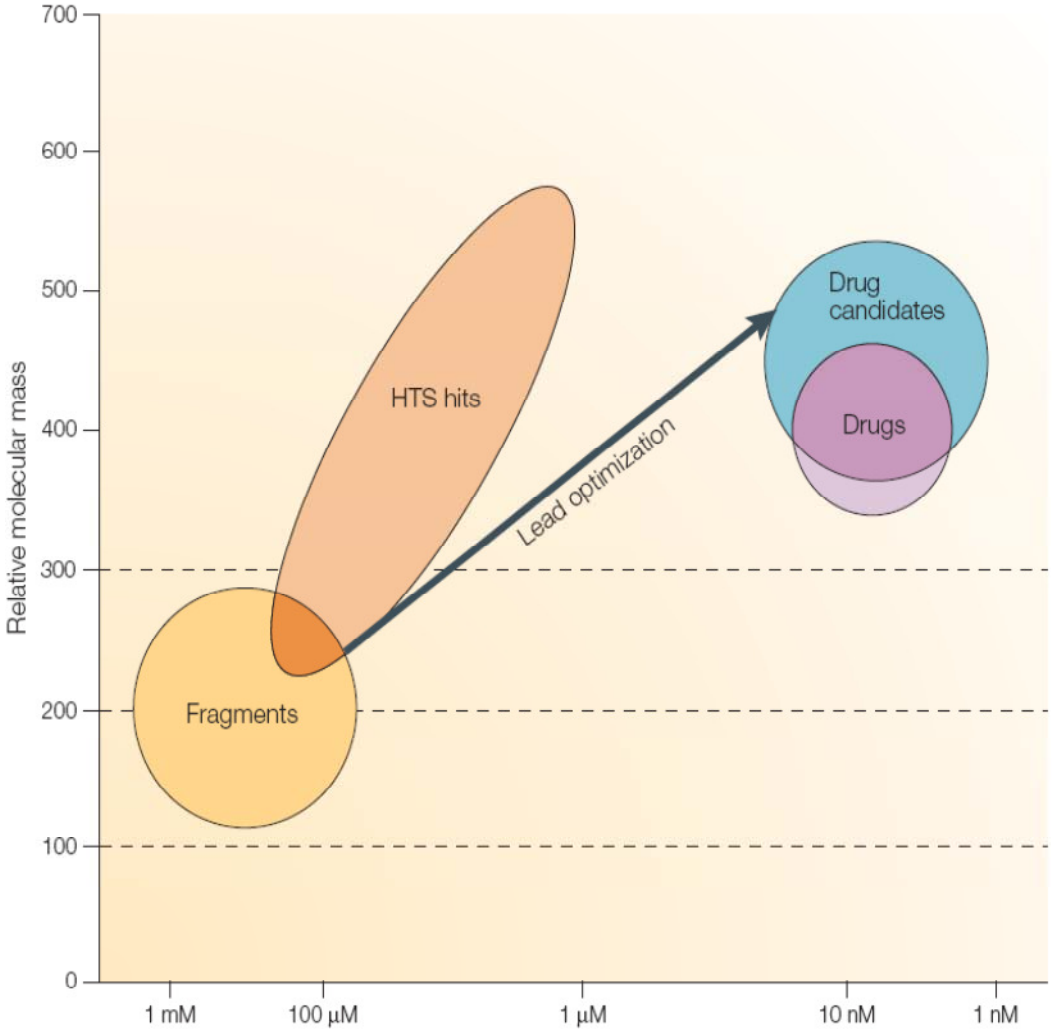


[1]Barker *et al.* (2010). Discovery of a Novel Hsp90 Inhibitor by Fragment Linking. *ChemMedChem* 5(10):1697-700.

Fragment screening is just the beginning

Structural insight on ligand binding required for optimisation

- Sensitive assay methods are necessary for fragment screening
  - E.g. 20K library on FCS++ (SPR, NMR, etc.)
- FBDD best suited to targets where protein X-ray crystal structures can be obtained
  - Rapid iterations by F/SBDD
- 'Build in' drug-like properties
  - Limit undesirable, excess features and ensure good solubility etc.
- Excellent coverage of chemical diversity
  - Novel start points with space for optimisation
- Challenge is in fragment-to-lead



D.C. Rees *et al., Nat. Rev. Drug Discov.*, 2004, 3, 660-672.

Fragment Development & General SBDD Strategies

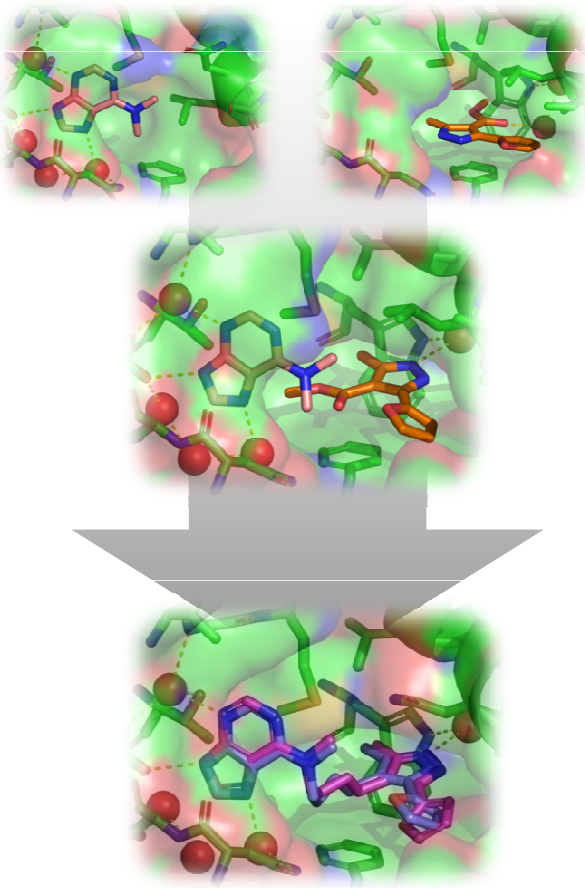
Evolution, searching, linking, & hybridization

Evolution → **Identify most promising hit**
- Ligand efficiency
- Confidence in binding mode
- Chemical expansion vector
- Synthetic tractability
- Secure anchoring points

SAR by nearest neighbour → **Search for commercial analogues**
(ABC - 16M library at Evotec)
- Purchase and test

Fragment Linking → **ID of adjacent fragments**
- Adjacent fragments can be linked
- Maintenance of interactions and poses and reduction of strain energies

Hybrid with existing leads → **3D Overlay with existing leads**
- Design by visual inspection
- Scaffold hopping and linker search tools

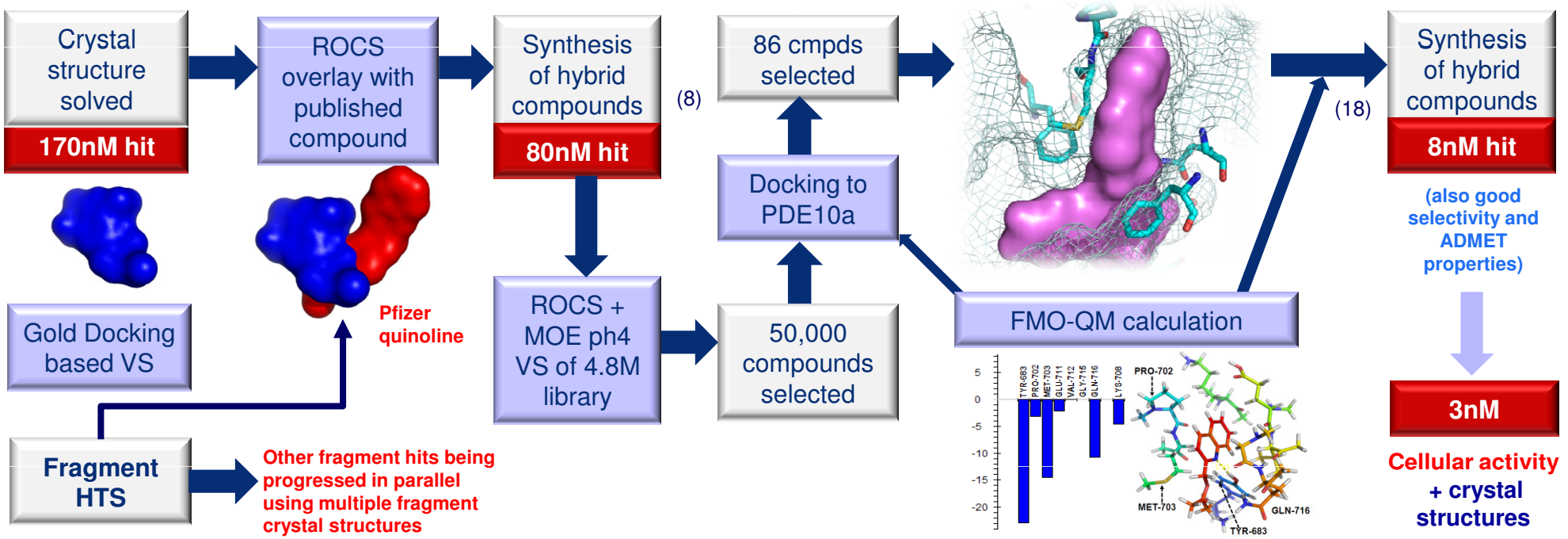


Hsp90 fragment linking example[1]

[1]Barker *et al.* (2010). Discovery of a Novel Hsp90 Inhibitor by Fragment Linking. *ChemMedChem* 5(10):1697-700.

Use of ROCS, docking, & FMO calc. in combined fragment, ligand & structure based design

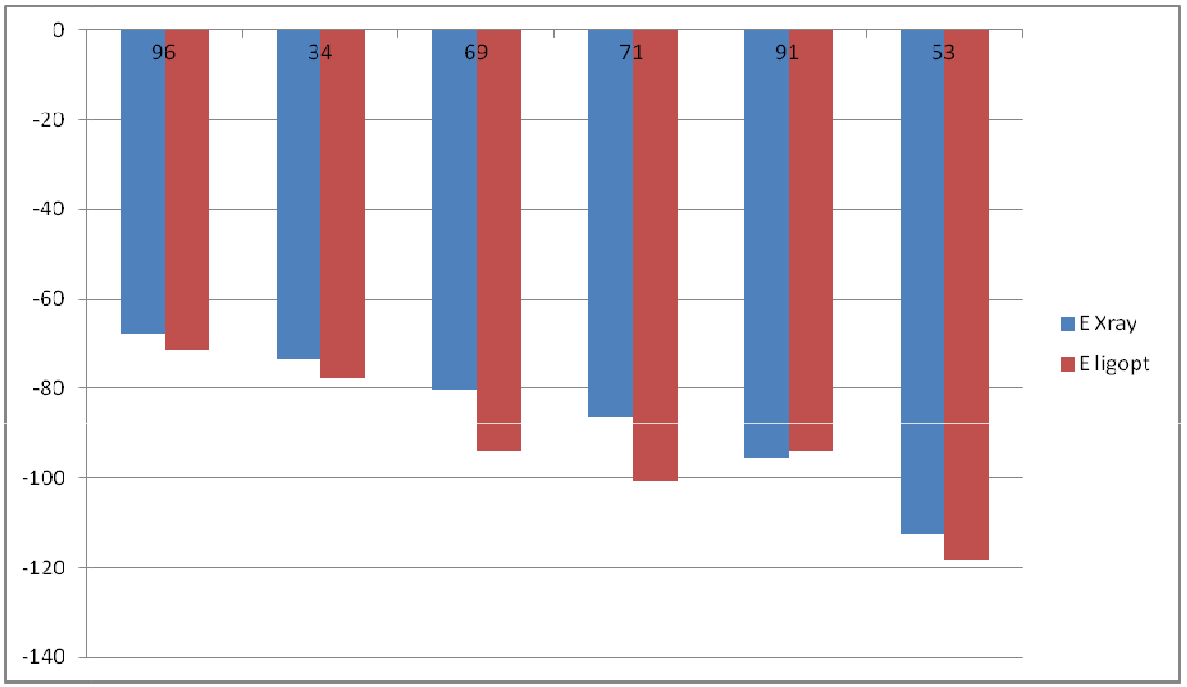
PDE10a case example



- Multiple computational methods used in tandem to guide medicinal chemistry
- Fragment structures and comp. chem. used in parallel to drive targeted hit-to-lead
- FMO QM calculation enabled precise filtering and potent & selective compound design

- FMO total energy of the ligand before and after ligand optimization

Complex	E Xray	E lig_opt
3569	-80.294	-93.907
3571	-86.376	-100.816
7091	-95.53	-94.119
7034	-73.628	-77.548
7053	-112.436	-118.318
6196	-67.853	-71.408
6196 (t2)	-61.849	-65.556

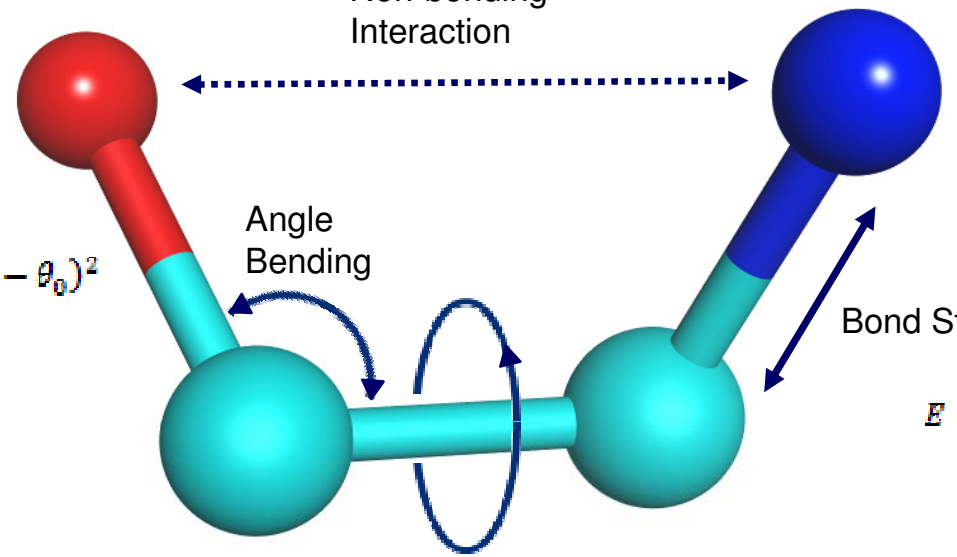


How MM (Molecular Mechanics) works

Energy terms and their basic functional forms

$$E = \left(\sum_i \sum_j \frac{-A_{ij}}{r_{ij}^6} + \frac{B_{ij}}{r_{ij}^{12}}\right) + \left(\sum_i \sum_j \frac{q_i q_j}{r_{ij}}\right)$$

Non-bonding Interaction



$$E = \sum k_\theta (\theta - \theta_0)^2$$

Angle Bending

Bond Stretching

$$E = \sum k_b (r - r_0)^2$$

Torsion

$$E = \sum A[1 + \cos(n\tau - \phi)]$$

Example of MM parameter file (MMFF94) for bond stretching

493 entries!!

*	types		kb	r0	Source
0	1	1	4.258	1.508	C94
0	1	2	4.539	1.482	C94
0	1	3	4.190	1.492	C94
0	1	4	4.707	1.459	X94
0	1	5	4.766	1.093	C94
*					
*					
*					
*					
0	76	76	4.286	1.357	X94
0	76	78	6.824	1.345	X94
0	78	78	5.573	1.374	C94
0	78	79	8.890	1.287	E94
0	78	81	5.046	1.381	C94
0	79	79	6.408	1.269	E94
0	79	81	4.305	1.356	E94
0	80	81	8.237	1.335	C94
$					

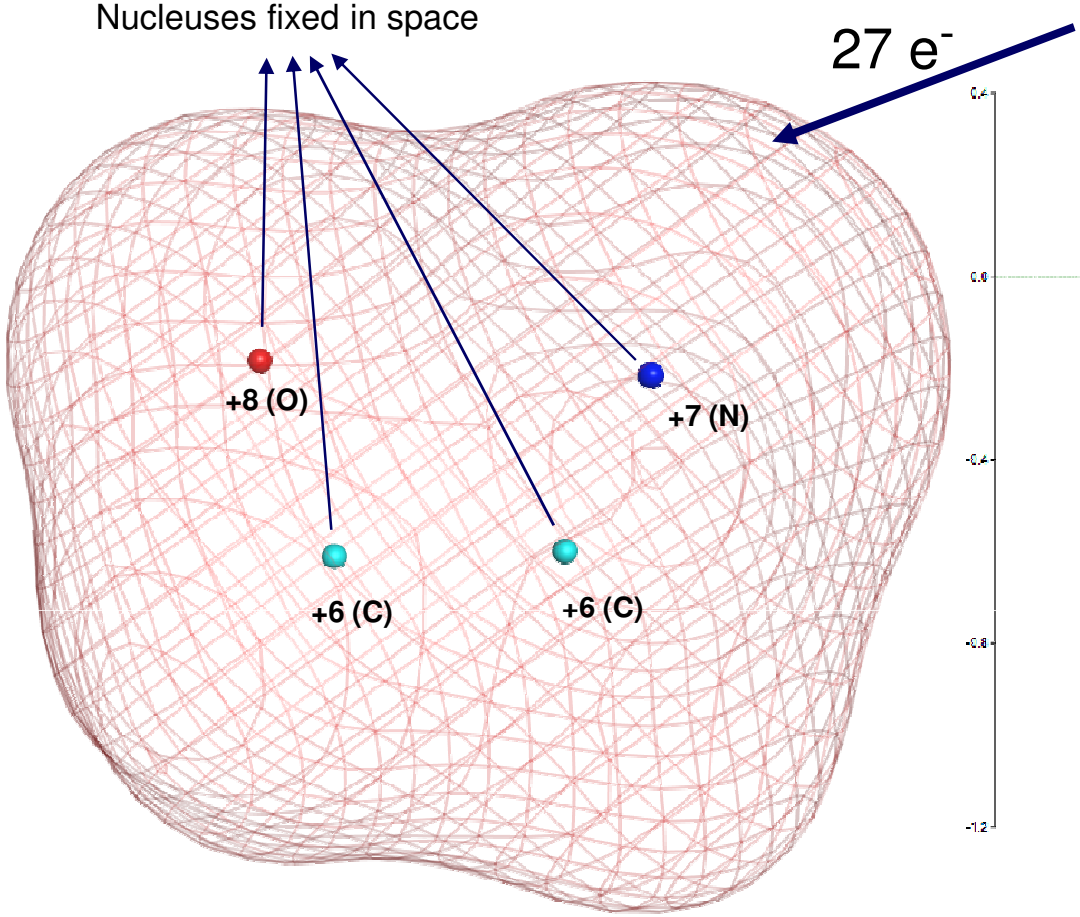
All the parameters are set to best reproduce experimental /ab initio QM results for exhaustive combinations of atom types

Hypothetical molecule: N-C-C-O

How QM (Quantum Mechanics) works

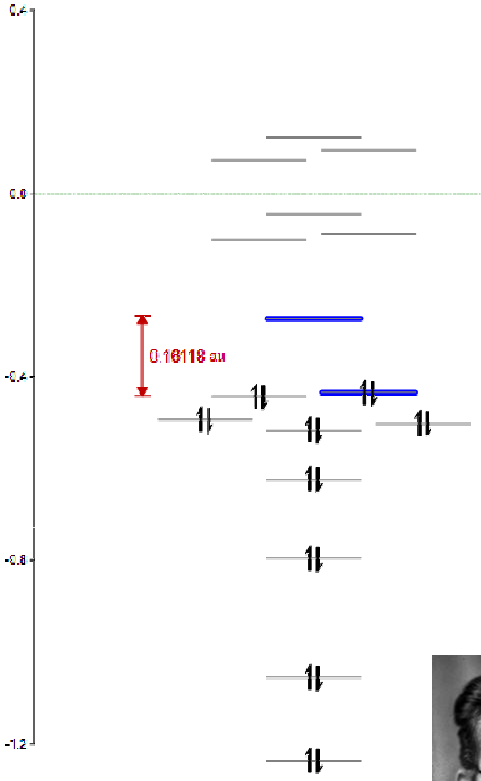
QM determines electronic states

Nucleuses fixed in space



Hypothetical molecule: N-C-C-O

Electrons are placed around four nulceuses



Parameters: Mass of electron, 9.11×10^{-31} kg
Plank's constant, 6.63×10^{-34} Js
Electric charge, -1.60×10^{-19} C

- No bonding information is used
- Distribution of 27 electrons around 4 nucleuses and the discrete energy levels (molecular orbitals) are calculated using Schrödinger's equation


evotec

```
ERROR: stackunderflow
OFFENDING COMMAND: ~

STACK:
```