Electrostatic Complementarity™, a Powerful tool for Drug Design: Optimizing Binding and Selectivity of Protein-Ligand Complexes

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Outline

> Introduction
> Electrostatic Complementarity (EC) calculations
> Case studies
  > Series of XIAP inhibitors - EC and activity correlation
  > Series of mGLU5 negative allosteric modulators - molecular design
  > Imatinib - selectivity
> Summary and future outlook
Flare™: Structure-based design

> Electrostatic Complementarity (EC)
  > Scoring
  > Visualization

> Protein Interaction Potentials
  > Gain vital knowledge of protein and ligand electrostatics to improve new molecule design

> 3D-RISM using XED or AMBER force fields
  > Understand the locations and stability of water in your protein

> WaterSwap analysis
  > Find the energetic hotspots in your protein

> Ligand focus
  > Standard physicochemical properties on every design
  > Add data from bespoke models or services
  > Multi-parametric scoring
  > Filtering using properties or sub-structure

> Accurate docking using Lead Finder™
  > Excellent reproduction of protein-ligand poses
  > Ensemble docking for handling active site flexibility

> Python® scripting
  > Most functions scriptable using Python
  > pyflare binary provides command-line access
  > Python examples and extensions available
  > UI customization
  > RDKit integration
  > Jupyter® Notebook integration
Electrostatic interactions and complementarity

> Electrostatic interactions between ligands and their receptors is an important factor (e.g., H-Bonding, ionic, cation-\(\pi\), \(\pi\)-\(\pi\), lone-pair-sigma hole (halogen-bonding) & orthogonal multipolar interactions (e.g., Fluorine bonding).
  > Molecular recognition
  > Binding free energy

> Assessing Electrostatic Complementarity (EC)
  > Insight of why ligand bind
  > Inform molecular design
  > Predict activity
Cresset electrostatics: The XED molecular mechanics force field

> eXtended Electron Distributions – ‘XED’
  > Multipoles via additional monopoles

> Huckel
  > separation of \(\pi\) and \(\sigma\) components of partial charges
    > \(\pi\) charges added to ‘xed’ atoms
    > \(\sigma\) charges added to nuclei
    > Excellent modeling of substituent effects
  > find bond orders and assign hybridization
    > Analogue N(sp\(^3\)) atoms – pyramidal to planar

> Full molecular mechanics force field with excellent coverage of organic chemistry, water and proteins
  > Minimization, conformations etc.
  > Not a dynamics force field

Ligand and protein molecular interaction potentials

2006 →
- Virtual screening
- Scaffold hopping
- R-group selection
- SAR analysis
- Molecule design

2017 →

Positive potential

Negative potential
Biotin-Streptavidin electrostatics

XED ESP surface of Streptavidin

XED ESP surface of Biotin

180° rotation

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) = \(1 - \frac{\text{ESP}_{\text{ligand}} + \text{ESP}_{\text{protein}}}{\text{MAX} (\text{ESP}_{\text{ligand}}, \text{ESP}_{\text{protein}})}\)
6. Color vertices according to complementarity
   → perfect electrostatic complementarity = 1 (green)
   → both potentials zero = 0 (white)
   → perfect electrostatic clash = -1 (red)
Biotin-Streptavidin electrostatics

XED ESP surface of Streptavidin

180° rotation

XED ESP surface of Biotin

Positive potential

Negative potential
Biotin-Streptavidin Electrostatic Complementarity

EC surface of Streptavidin

EC surface of Biotin

180° rotation

Good EC

Poor EC
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)
Application to a series of XIAP inhibitors

> 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

| Table 2. XIAP-BIR3 Affinity of Substituted Indolines 7–16 |
|---|---|---|---|---|
| compd | R | Hammett $\rho$ | XIAP-BIR3 EC$_{50}$ (nM) or %I | XIAP-BIR3 LE $^{(2)}$ (kcal mol$^{-1}$ per non-H atom) |
| 7 | –H | 0.00 | 52% 495 nM | –0.24 |
| 8 | –NO$_2$ | –0.46 | 56% 1500 nM | –0.20 |
| 9 | –OMe | –0.27 | 49% 155 nM | –0.25 |
| 10 | –Me | –0.17 | 46 | 0.30 |
| 11 | –F | –0.15 | 59 | 0.26 |
| 12 | –P | 0.00 | 51 | 0.29 |
| 13 | –Cl | 0.23 | 13 | 0.33 |
| 14 | –Br | 0.23 | 9.8 | 0.34 |
| 15 | –CF$_3$ | 0.54 | 3.9 | 0.31 |
| 16 | –SO$_2$Me | 0.72 | 4.1 | 0.32 |

$^{(2)}$Values were determined by fluorescence polarization assay (see Experimental Section). Potency data are reported as the mean of at least two runs.

$^{(2)}$Values calculated according to the Hipsley formula.  

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> 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

Increase electron withdrawing effect

Compound 8  
PIC50 = 3.0

Compound 7  
3.3

Compound 10  
4.3

Compound 13  
4.5

Compound 17  
5.1

Increase electron withdrawing effect
Electrostatic Complementarity of five XIAP inhibitors

- Compound 8
  - pIC$_{50}$ = 3.0
  - SCI = 0.17
  - R = 0.25
  - Rho = 0.23

- Compound 7
  - pIC$_{50}$ = 3.3
  - SCI = 0.2
  - R = 0.33
  - Rho = 0.32

- Compound 10
  - pIC$_{50}$ = 4.3
  - SCI = 0.2
  - R = 0.32
  - Rho = 0.32

- Compound 13
  - pIC$_{50}$ = 4.9
  - SCI = 0.24
  - R = 0.38
  - Rho = 0.37

- Compound 17
  - pIC$_{50}$ = 5.1
  - SCI = 0.25
  - R = 0.36
  - Rho = 0.41
EC to XIAP binding site

> 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\textsubscript{50} correlation for the XIAP series

> Nice correlation between the XIAP-BIR3 pIC\textsubscript{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

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

Table 1. In Vitro Profile of Compounds 6–17

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>R1</th>
<th>R2</th>
<th>mGlu8 pIC50</th>
<th>mGlu8 pIC50</th>
<th>RLM 50 (min)</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>F</td>
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<td>6.4</td>
<td>22</td>
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<tr>
<td>7</td>
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<td>N</td>
<td>C</td>
<td>F</td>
<td>6.6</td>
<td>nd</td>
<td>6</td>
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<tr>
<td>8</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>H</td>
<td>6.4</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>F</td>
<td>5.3</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>10</td>
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<td>OMe</td>
<td>H</td>
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<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
<td>N</td>
<td>CONH</td>
<td>H</td>
<td>4.2</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>N</td>
<td>CONH</td>
<td>H</td>
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<td>7.9</td>
<td>30</td>
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<tr>
<td>13</td>
<td>N</td>
<td>N</td>
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<td>C</td>
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<td>8.3</td>
<td>32</td>
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<tr>
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<td>H</td>
<td>C</td>
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<tr>
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<td>CN</td>
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<td>N</td>
<td>C</td>
<td>H</td>
<td>7.2</td>
<td>7.1</td>
<td>32</td>
</tr>
</tbody>
</table>

*nd = not determined.

Table 2. In Vitro Profile of Compounds 21–30

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>R1</th>
<th>R2</th>
<th>mGlu8 pIC50</th>
<th>mGlu8 pIC50</th>
<th>RLM 50 (min)</th>
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</thead>
<tbody>
<tr>
<td>21</td>
<td>CH</td>
<td>CH</td>
<td>H</td>
<td>8.5</td>
<td>8.6</td>
<td>43</td>
</tr>
<tr>
<td>22</td>
<td>CH</td>
<td>CH</td>
<td>F</td>
<td>8.9</td>
<td>8.8</td>
<td>31</td>
</tr>
<tr>
<td>23</td>
<td>CH</td>
<td>CMe</td>
<td>H</td>
<td>8.6</td>
<td>8.3</td>
<td>19</td>
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<tr>
<td>24</td>
<td>CH</td>
<td>CF</td>
<td>H</td>
<td>8.8</td>
<td>8.6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>25</td>
<td>CH</td>
<td>CF</td>
<td>H</td>
<td>9.3</td>
<td>9.2</td>
<td>44</td>
</tr>
<tr>
<td>26</td>
<td>CH</td>
<td>CONH</td>
<td>H</td>
<td>9.2</td>
<td>9.2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>27</td>
<td>CH</td>
<td>CH</td>
<td>H</td>
<td>8.8</td>
<td>8.5</td>
<td>35</td>
</tr>
<tr>
<td>28</td>
<td>CH</td>
<td>CF</td>
<td>F</td>
<td>9.1</td>
<td>9.4</td>
<td>87</td>
</tr>
</tbody>
</table>
| 29 | N | N | H | 8.0         | 8.7         | nd*
| 30 | CH | N | H | 8.3         | 7.5         | nd*          |

*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
Impact of fluorination on EC and activities

**A**

- **Compound 13**
  - $pKi = 8.4$
  - $SCI = 0.40$
  - $R = 0.57$
  - $Rho = 0.52$

- **Compound 17**
  - $pKi = 7.7$
  - $SCI = 0.38$
  - $R = 0.47$
  - $Rho = 0.48$

**B**

- **Compound 21**
  - $pKi = 8.5$
  - $SCI = 0.40$
  - $R = 0.58$
  - $Rho = 0.56$

- **Compound 25**
  - $pKi = 9.3$
  - $SCI = 0.46$
  - $R = 0.62$
  - $Rho = 0.61$
Imatinib: EC and selectivity

<table>
<thead>
<tr>
<th>Target</th>
<th>PDB</th>
<th>pKD</th>
<th>Complem.</th>
<th>Complem. r</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-SRC</td>
<td>2D1Q</td>
<td>4.4</td>
<td>0.36</td>
<td>0.55</td>
</tr>
<tr>
<td>p38α</td>
<td>3HEC</td>
<td>5.5</td>
<td>0.41</td>
<td>0.51</td>
</tr>
<tr>
<td>SYK</td>
<td>1XBB</td>
<td>5.5</td>
<td>0.27</td>
<td>0.43</td>
</tr>
<tr>
<td>ANC-AS</td>
<td>4CSV</td>
<td>6.0</td>
<td>0.32</td>
<td>0.40</td>
</tr>
<tr>
<td>LCX</td>
<td>2PL0</td>
<td>7.4</td>
<td>0.32</td>
<td>0.43</td>
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<tr>
<td>KIT</td>
<td>1T46</td>
<td>7.9</td>
<td>0.33</td>
<td>0.50</td>
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<td>0.36</td>
<td>0.48</td>
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<tr>
<td>ABL2</td>
<td>3GVU</td>
<td>8.0</td>
<td>0.43</td>
<td>0.57</td>
</tr>
<tr>
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<td>1OPI</td>
<td>9.0</td>
<td>0.43</td>
<td>0.63</td>
</tr>
<tr>
<td>DDR1</td>
<td>48KJ</td>
<td>9.1</td>
<td>0.41</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* Imatinib binding decreased due to conformational penalty upon binding
Application to additional data sets

TYK2

R² = 0.5933

ΔG (kcal/mol)

RPA70N

R² = 0.3001

Kd (µM)

PERK

R² = 0.7937

IC50 (nM)

mGlu5

R² = 0.68

pKi

XIAP

R² = 0.6913

IC50 (µM)

DPP4

R² = 0.5685

pIC50

MCL1

R² = 0.6583

dG (kcal/mol)

Complementarity; Complementarity r; Complementarity rho
Summary and future outlook

> Meaningful assessment of electrostatic complementarity at low computational cost (< 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
Recent publication

M.R. Bauer and M.D. Mackey

Electrostatic Complementarity as a Fast and Effective Tool to Optimized Binding and Selectivity of Protein-Ligand Complexes

J. Med Chem. 2019, 62, 3036-3050
Thank you. Questions?

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