Structure-Based drug design

How can we best use the data base of three-dimensional structures to help us more efficiently design drugs?

* X-ray Crystallography- HIV protease inhibitor
* NMR Spectroscopy- SAR by NMR
* Computational modeling
* Ligand binding sites in macromolecules
* Combinatorial chemistry - Huge libraries of small molecules.
Role of X-ray crystallography

* Capable of providing very high resolution structures which are needed to determine precise atomic level descriptions of ligand binding sites.

* Things often crystallize better in the presence of ligand as a result of increased stability (less floppy regions).

* Once crystallization techniques have been worked out for one complex, they should be fairly similar with subsequent complexes.

* Well-suited for studying small samples of molecules that have been screened by a previous method but not really suited for library screenings.
Role of NMR spectroscopy

* One is not always able to get high enough resolution needed for drug design.
* Limited to smaller macromolecules, more or less 30 kDa or less.
* It is a method that is capable of determining the position of some hydrogen bonds.
* It is suited for rapid screening of large number of molecules as potential ligands using SAR by NMR.
* It can be very quick at mapping residues that are altered following ligand binding.
Why is it so hard?

* Proteins exist in many different conformational states which influence and are influenced by ligand binding.
* A single fixed protein structure represents only a very narrow window for ligand binding.
* We must learn how to accommodate loop fluctuations and domain movement in our design regimens.
* We must learn how to deal with solvents and electrostatic interactions if we hope to make more accurate predictions of binding strengths.
SAR by NMR

* NMR-based screening method.
* Uses a simple (quick) experiment to identify small organic molecules that bind to proximal subsites of a protein.
* The molecules are then linked together to produce high-affinity ligands.
* This can be extremely powerful when used with small molecule libraries to identify lead-compounds

Outline of SAR by NMR

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\[ \Delta G = \Delta G_A + \Delta G_B \]
SAR by NMR applied to Bcl-x<sub>L</sub>

- Bcl (B-Cell Lymphoma) family of proteins plays a key role in maintenance of normal cellular homeostasis.
- Overexpression leads to oncogenic transformations and plays a role in drug resistance in certain forms of cancer.
- The family of protein consists of both antiapoptotic (Bcl-2, Bcl-xL) and proapoptotic (Bak, Bax, Bad) members.
- The structure of several family members is known.
- The structure consists of two hydrophobic helices surrounded by 5 to 7 amphipathic helices.
- The antiapoptotic members have a groove that binds to an α-helix (BH3) present in the proapoptotic members.

Identification of first Bcl-\(x_L\) site.

- Performed NMR based screen to identify molecules that would compete with binding of proapoptotic proteins to Bcl-\(x_L\).
- Identified that several biaryl compounds bound to the same binding pocket of Bcl-\(x_L\) as proapoptotic proteins.
- These compounds bind in the same position as a conserved leucine residue in the BH3 helice
- This served as first binding site for SAR by NMR protocol applied to Bcl-\(x_L\).

Affinities of selected biaryl compounds to Bcl-\textsubscript{xL}

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>NMR K\textsubscript{d} (\textmu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>$300 \pm 30$</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>$1200 \pm 530$</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>$&gt; 5000$</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>$2000 \pm 1600$</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>$1990 \pm 990$</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>$383 \pm 117$</td>
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<tr>
<td>9</td>
<td><img src="image9.png" alt="Structure" /></td>
<td>$238 \pm 110$</td>
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<tr>
<td>10</td>
<td><img src="image10.png" alt="Structure" /></td>
<td>$250 \pm 139$</td>
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</table>

Biaryl acid bound to Bcl-\(x_L\)

Black: HSQC of \(^{15}\text{N}-\text{Bcl-}x_L\).

Red: HSQC of \(^{15}\text{N}-\text{Bcl-}x_L\) with biaryl acid (Compound 1).

Identification of first Bcl-x$_L$ site.

- Performed additional NMR based screen to identify molecules that would bind to a different region of Bcl-x$_L$.
- Identified that several aromatic compounds that bound to and adjacent binding pocket of Bcl-x$_L$ as proapoptotic proteins.
- These compounds bind in the same position as a conserved isoleucine residue in the BH3 helice.
- This served as second binding site for SAR by NMR protocol applied to Bcl-x$_L$.

## Affinities of selected second site Bcl-\(x_L\) binders

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>NMR (K_d) ((\mu)M)</th>
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<tbody>
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<tr>
<td>12</td>
<td><img src="Image" alt="Structure 2" /></td>
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<tr>
<td>13</td>
<td><img src="Image" alt="Structure 3" /></td>
<td>5000 ± 2000</td>
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<tr>
<td>14</td>
<td><img src="Image" alt="Structure 4" /></td>
<td>2000 ± 440</td>
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<tr>
<td>15</td>
<td><img src="Image" alt="Structure 5" /></td>
<td>11000 ± 4800</td>
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<tr>
<td>16</td>
<td><img src="Image" alt="Structure 6" /></td>
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<tr>
<td>17</td>
<td><img src="Image" alt="Structure 7" /></td>
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<td>18</td>
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<td>19</td>
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<tr>
<td>20</td>
<td><img src="Image" alt="Structure 10" /></td>
<td>6000 ± 2000</td>
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</tbody>
</table>

Napthol analog bound to Bcl-x<sub>L</sub>

Black: HSQC of $^{15}$N-Bcl-x<sub>L</sub>.

Red: HSQC of $^{15}$N-Bcl-x<sub>L</sub> with biaryl acid (Compound 1).

Green: HSQC of $^{15}$N-Bcl-x<sub>L</sub> with napthol (Compound 11).

SAR by NMR applied to Bcl-xL

SAR by NMR applied to Bcl-\textsubscript{x}\textsubscript{L}

SAR by NMR applied to Bcl-x_L

SAR by NMR applied to Bcl-x\textsubscript{L}

Affinities of acylsulfonamides to Bcl-x\textsubscript{L}

![Chemical structure of acylsulfonamide](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>NMR K\textsubscript{d} (\textmu M)</th>
<th>Bcl-x\textsubscript{L} FPA K\textsubscript{i} (\textmu M)</th>
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</thead>
<tbody>
<tr>
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<td>Me</td>
<td>320 ± 35</td>
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<tr>
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</tbody>
</table>

SAR by NMR applied to Bcl-\(x_L\)

![Diagram of SAR by NMR applied to Bcl-\(x_L\)]

Structure of compound 31 bound to Bcl-xL

SAR by NMR applied to Bcl-x<sub>L</sub>