Trip Report: Structure Based Drug Discovery Whistler, British Columbia, Canada April 4 -9, 2006

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***Abstract**: The Keystone Symposium was held in Whistler, British Columbia, Canada, April 4-9, 2006. The symposium consisted of four main sections: "High-Throughput Elucidation of Structural Information", "Structural Chemistry of Human Protein Families", "FAST Lead Discovery", and "A Site-Directed Mutagenesis Approach to Define Drug Binding Sites on hERG. This report highlights selected material from the seminars.*

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"High-Throughput Elucidation of Structural Information"
Andrzej Joachimia, Argonne National Laboratory, Argonne, IL.

Dr. Joachimiak presented a seminar on the use of the "Advanced Photon Source (APS)" synchrotron at the Department of Defense Argonne National Laboratory as a provider of excellent X-ray beams for performing protein crystallography. The synchrotron generates extremely high quality X-ray beams with very low diversion, which gives two distinct advantages over regular X-ray sources; resolution of crystal structures and time taken to acquire the crystal structure. The information you can glean from an x-ray structure is heavily dependent upon the resolution, which is possible down to 0.8 Å using the synchrotron X-ray source.

2 Å resolution – can distinguish peptides, but not individual atoms
1.2 Å resolution – can determine interatomic distances and solvent structure
0.8 Å resolution – possible to determine protonation states as well as bond densities

This degree of resolution enables a very fine description of the state of a protein and interaction of potential drugs in binding sites.

Atomic resolution structures reveal –

- Multiple conformations
- Chemical modifications
- Unexpected stereochemistry
- Distortion of bond lengths
- Protonation events
- Ligand binding details

Some minor drawbacks are the susceptibility of the protein to radiation damage with such high powered focused X-rays. Typically, samples are cryo-cooled in liquid helium to prolong crystal life and also reduce background noise. This is also very expensive technology, costing $1M for 1 hour per week of beam time.

"Structural Chemistry of Human Protein Families"
Aled Edwards, University of Toronto, Toronto, ON, Canada.

Dr. Edwards presented a seminar on the work of the Structural Genomics Corporation. The SGC is a public/private partnership which was formed to determine the 3D structures of proteins of relevance to human health. The consortium has labs based in Toronto, Oxford and Stockholm and has been operational since July 2004. In 2005, the SGC deposited >150 structures into the Protein Data Bank, accounting for ~20% of all structures deposited. Over 50% of the structures determined by the SGC were enabled by the addition of a small molecule, derived either from the literature or using in-house screens against focused libraries. The SGC is currently on target to reach their goals of achieving a deposition rate of ~200 structures per year and at a cost basis of $125,000/structure.

The success of the small molecule efforts prompted the SGC to investigate experimental computational programs for understanding the structural chemistry of human gene families, and to explore the structural basis of selectivity and specificity in proteins which are generally highly conserved. This work is currently on-going.

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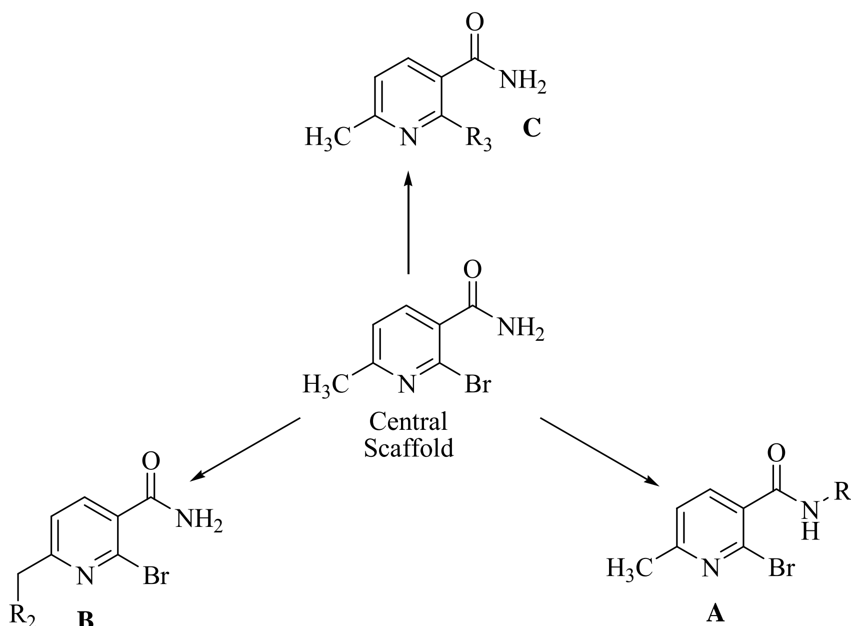
"FAST Lead Discovery"
Jeff Blaney,SGX Pharmaceuticals, Inc., San Diego, CA.

This presentation focused on the proprietary drug discovery platform developed by SGX. This approach, known as FAST (*Fragments of Active Structures*) uses high throughput X-ray crystallography screening to detect, visualize and identify small ligands that are bound to the target protein. The small ligand libraries used in this approach have molecular weights in the 150-200 range and are amenable to rapid chemical elaboration, generally possessing two to three points of chemical diversity. The nature of the chemical libraries used generate hits from screening that are generally classified as "lead-like", rather than "drug-like" as they are highly amenable to further modification and optimization. An example of a lead-like library used for screening is shown in Figure 1. Initial lead optimization is performed using knowledge of the co-crystal structure and computational methods to generate small focused (one dimensional) libraries (**A-C**) which are evaluated with *in-vitro* biochemical assays as well as co-crystal structure determinations. Optimal variants at each point of diversity are then combined to synthesize two- or three dimensional focused libraries, which are evaluated again with assays and crystallography. Further optimization is then performed using traditional medicinal chemistry techniques.

Figure 1



An example of the use of the FAST approach, targeting Gleevec resistant BCR-ABL mutants was presented. Initial fragment hits were in the order of ~10 μM, which upon optimization using the approach described above was increased to 4 nM. With using the combination of *in-vitro* assays and co-crystal structural determinations, they were able to find that their compound which was active against the BCR-ABL Gleevec resistant mutant, was binding the active form of the enzyme. This is in contrast to Gleevec, which binds the inactive form of the enzyme.

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"A Site-Directed Mutagenesis Approach to Define Drug Binding Sites on hERG"
Michael Sanguinetti, Univeristy of Utah, Salt Lake City, UT.

One of the most recognized hurdles for successful drug development is the avoidance of drug-induced cardiac arrhythmias. Of this, the most common cause is QT interval prolongation, which is caused by drugs that block the hERG (human Ether-a-go-go related gene) potassium channels and cause a delay in re-polarization of the ventricular monocytes. Since the discovery of hERG, a number of drugs have been pulled from the market due to these drug-induced arrhythmias. There is currently no structure for hERG available, but it is known to be formed by the assembly of four subunits, which each contain four transmembrane α-helical domains.

hERG blockers are generally structurally diverse. However, potent blockers typically contain a basic nitrogen at the centre of the molecule and aromatic groups positioned at the ends. Typically, the drugs that block hERG gain access from the cytoplasm and block the open pore and hence block K^+ flux. Site-directed mutagenesis experiments revealed one particular region of hERG thought to be responsible for binding. This is located in the S6 region, towards the central cavity and forms a variable binding site which is only accessible when the channel is in an open state. The mutagenesis experiments reveal that several drugs form hydrophobic interactions with Phe-656, a cation-π interaction with Tyr-652 and H-bonding polar interactions with Thr-623 and Ser-624. Comparing sequence homologies of hERG with other K^+ channels in this S6 region, reveals a possible reason why hERG is so promiscuous in the compounds it binds. An example being Kv1.4 whose sequence at this particular region is *I*ALP*V*, compared to hERG which is *Y*ASI*F*. The main differences highlighted by italics show the presence of the tyrosine and phenylalanine residues, responsible for the aromatic binding, compared to the isoleucine and valine in the Kv1.4.

There are a number of proposed pharmacophore models that have been described for the hERG binding site and they can be found in the following references (Cavalli *et al. J. Med. Chem.* **2002**, *45*, 3844, Farid *et al. Bioorg. Med. Chem.* **2006**, *14*, 3160).

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