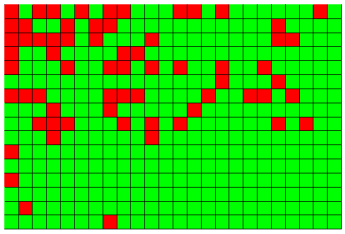
Mapping and Docking of GPCR-Drug Interactions using Shotgun Mutagenesis

Mapping Drug-Target Interactions

Direct visualization of molecular interactions, such as by co-crystallography or NMR, is increasingly being used to guide drug discovery, at least for soluble proteins. Accurate structural models are useful for determining the mechanism by which drug candidates bind their targets, for chemical optimization of lead compounds, for *in silico* drug screening, and for *de novo* drug design. However, integral membrane proteins, such as GPCRs and ion channels, are not typically amenable to manipulation for direct structural analysis. Instead, molecular interactions of membrane proteins are often investigated by site-directed mutagenesis, where the involvement of amino acids in a binding event are inferred after assaying the functional effects of point mutations. This process, while effective, is laborious and slow, requiring cloning, expression, and individual testing of hundreds or thousands of point mutations for complete analysis of a typical protein. New high-throughput mapping approaches applicable to membrane proteins will provide the ability to routinely obtain important structural information about these important drug targets.

Shotgun Mutagenesis

Integral's Shotgun Mutagenesis approach rapidly generates detailed maps of putative drug binding sites on membrane proteins by high-throughput cell expression and analysis of individual point mutations. Clone libraries containing several thousand point mutants are expressed simultaneously and individually within eukaryotic cells and analyzed in high-throughput microplate formats using standard binding or functional assays. Every residue of a target protein is mutated several times, introducing both conserved and non-conserved substitutions, thereby allowing the effects of individual side-chain contact points to be investigated. The structural detail provided by Shotgun Mutagenesis can be used for guiding computational docking of drugs with their targets. Because whole libraries can be faithfully reproduced for repeated analysis, the binding sites for dozens of different drugs and ligands can be rapidly mapped on the same target and compared.



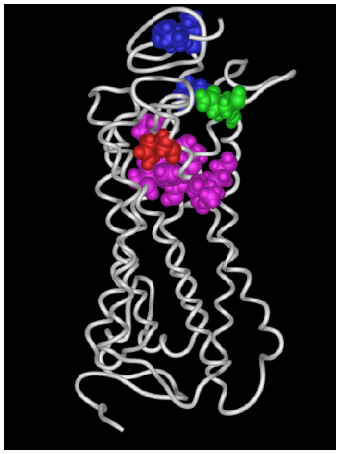


Figure 1. HIV-1 (JRFL) infection assays were performed in the presence and absence of TAK-779. Mutations that continued to support HIV infection even in the presence of TAK-779 were identified. Amino acid residues (in color) required for TAK-779 inhibition were mapped onto a three-dimensional model of CCR5.

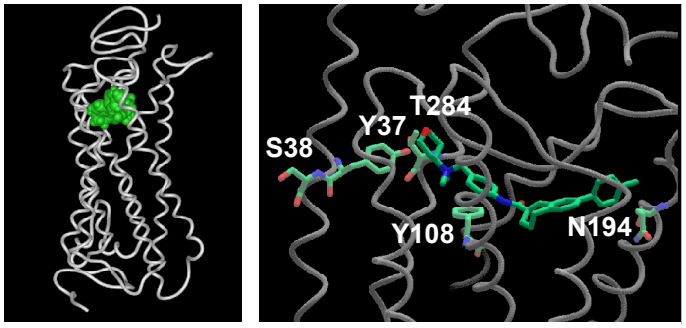


Figure 2. A model for TAK-779 binding to CCR5. TAK-779, shown in green, bound to a pocket formed by the transmembrane helices of CCR5, and in close proximity to five amino acid constraints identified by Shotgun Mutagenesis (white).

Technical Description

Shotgun Mutagenesis was used to map the binding site of a small-molecule inhibitor, TAK-779, on the GPCR and HIV coreceptor CCR5. A library of plasmids coding for point mutations along the entire 1 kb CCR5 gene was prepared, and each clone was individually expressed in mammalian cells. Surface expression and full-length translation of each clone were first tested using flanking epitope tags. Amino acids that are critical to inhibition of CCR5 by TAK-779 were then identified by cell infection assay using an HIV luciferase reporter virus. The TAK-779 binding site was mapped on CCR5 by identifying point mutations that continued to support HIV use of the coreceptor, but that eliminated inhibition by TAK-779 (Figure 1). Among the amino acids identified were a number that have been previously implicated in TAK-779 binding. Five putative direct-contact residues were then used as constraints to guide the computational docking of the TAK-779 molecule with the CCR5 model, by optimizing space fitting, electrostatic arrangements, van der Waals forces, and hydrogen bonding. The energy-minimized binding configuration provided a three dimensional model of the most plausible drug-target interaction (Figure 2). Shotgun Mutagenesis thus enables the routine identification of amino acids within membrane proteins that are important for drug binding, allowing the generation of high-quality structural models of drug-target interactions.

Contact Us

Shotgun Mutagenesis mapping, including comprehensive mutation of user-specified genes, data collection, and structural analyses, are provided to customers on a fee-for-service basis. For more information contact us at:

215.966.6061
info@integralmolecular.com
www.integralmolecular.com