

High-throughput Mapping of Structures

Shotgun Mutagenesis is a novel strategy for investigating protein structure-activity relationships by rapidly evaluating functional effects of point mutations across an entire target protein. Using a patented high-throughput expression method, thousands of point mutations of a target protein can be concurrently evaluated for functional protein activity. Shotgun Mutagenesis begins with the creation of a customized plasmid library for a target gene, each clone in the library bearing a unique amino acid mutation. Clones are individually arrayed in microplates and expressed within living mammalian cells. Each clone is validated for expression on the cell surface and for full-length translation to identify the most useful clones containing isolated amino acid substitutions (Figure 1). The entire library of clones is then simultaneously tested for a defined function of interest, such as antibody binding or agonist-induced signaling. Because each clone is sequenced at the time of library creation, amino acids critical for the function are readily identified by a loss of reactivity. These residues are mapped onto the protein structure to visualize functional moieties (Figure 2).

Advantages of Shotgun Mutagenesis

Shotgun Mutagenesis enables structural analyses of even difficult proteins, such as GPCRs, that require eukaryotic cells for proper expression, folding, and function, and whose structures cannot be routinely analyzed by direct methods such as crystallography or NMR. Structure-activity relationships determined by conventional site-directed mutagenesis can require years to map even fractions of a protein's topography. By comparison, Shotgun Mutagenesis enables comprehensive analysis of every amino acid in the protein within weeks of initiating an experiment. Expressed libraries can be analyzed using any microplate-based cellular assay, allowing diverse protein interactions and functions to be mapped. Integral Molecular has developed customized software for tracking and analyzing the tens of thousands of data points involved in array production, validation, and experimentation (Figure 3).

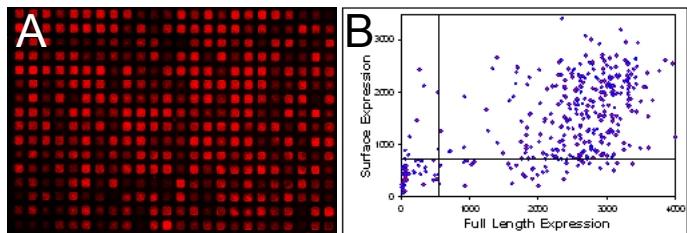


Figure 1. Validation of libraries **A.** Each clone is verified for cell surface expression and full length translation. Wild type clones and non-specific proteins are also included on each plate as controls. **B.** Only verified clones are used for functional mapping analysis.

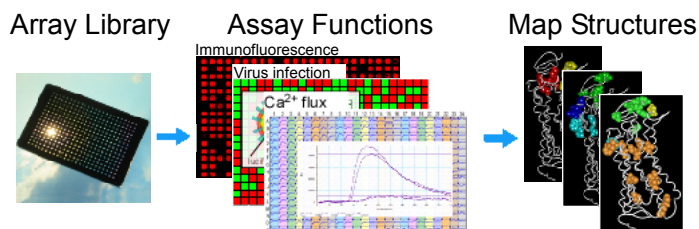


Figure 2. A library of mutated clones is arrayed for analysis. Replicate plates are tested using any functional assay adapted for cell-based analysis in microplates. Once analyzed, identified mutations are mapped onto protein structures.

Applications of Shotgun Mutagenesis

Shotgun Mutagenesis has been used to map diverse GPCR interactions, including the binding sites for antibodies, agonists, and small molecules. Antibody epitopes and drug binding pockets can be mapped to specific amino acids and, as desired, to specific atoms within amino acid side chains. These maps have direct utility for understanding the structural and functional regions of proteins, for protein modeling and *in silico* docking, and for making lead candidate and intellectual property decisions. Additional applications of Shotgun Mutagenesis include:

- Identifying membrane protein signaling motifs
- Optimizing the activity of secreted proteins
- Mapping functional regions of cytoplasmic proteins
- Identifying DNA/RNA active elements

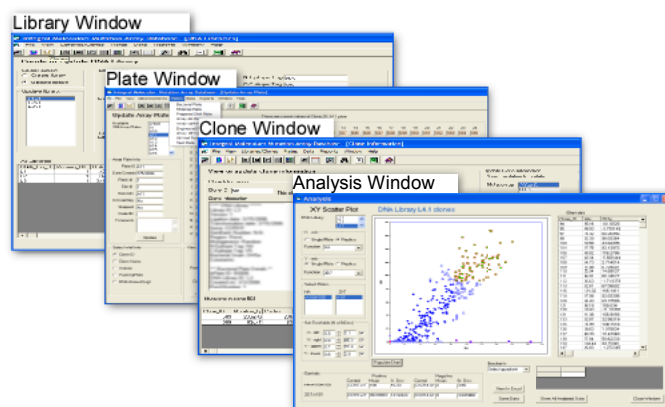


Figure 3. The tens of thousands of data points typically generated during library production, validation, and experimentation are tracked and analyzed using Integral Molecular's proprietary software.

Contact Us

Shotgun Mutagenesis analyses of protein interactions are provided to customers on a fee-for-service basis, which includes customized Shotgun Mutagenesis of user-specified genes, data collection, and structural analyses. For more information contact us at:

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