

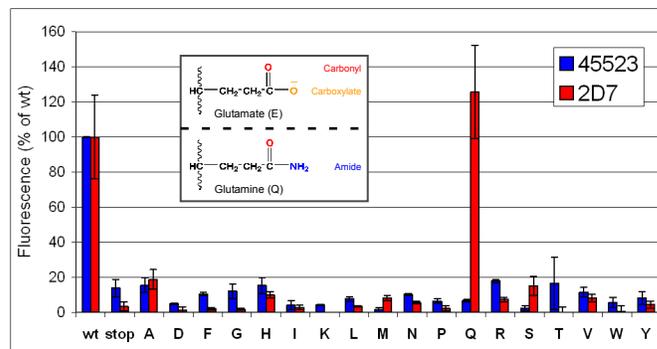
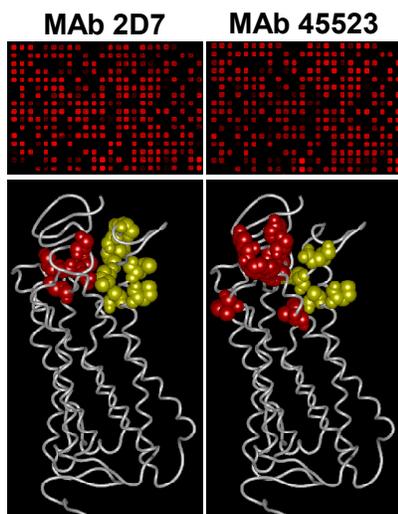
## Mapping of Antibody Binding Epitopes

Many useful monoclonal antibodies (MAbs) recognize conformationally complex protein epitopes. Direct methods for locating and distinguishing these epitopes, including co-crystallography, are often impractical and cannot readily be utilized for membrane protein targets such as GPCRs and ion channels. Identification and mapping of MAb epitopes is therefore commonly achieved using site-directed mutagenesis. Epitope mapping by this approach involves systematically introducing residue substitutions along the target protein, and then determining the effect of each on MAb recognition. Because each point-mutation must be individually sequence-verified, expressed in cells, and assayed for altered expression and reactivity, conventional mutational mapping is usually slow and laborious. Using a high-throughput "Shotgun Mutagenesis" approach to structure-function mapping, Integral Molecular is able to rapidly construct comprehensive epitope maps across the entire sequence of even difficult target proteins.

## Shotgun Mutagenesis

Shotgun Mutagenesis uses a proprietary high-throughput cell expression technology that enables the expression and analysis of large libraries of mutated target proteins within eukaryotic cells. Every residue in a protein is mutated, usually multiple times, in order to assay changes in function. Entire mutation libraries can be repeatedly expressed and assayed, making it possible to rapidly map the complete epitopes of dozens of different antibodies. Proteins are expressed within standard mammalian cell lines, so even difficult proteins that may not express or function properly in other cell types can be assayed. Proteins can be analyzed using any standard eukaryotic cell-based assay, such as immunofluorescence, enabling the role of every amino acid in MAb epitopes, or other protein functions, to be mapped.

**Figure 1.** Binding of CCR5 MAbs to a 384-well microplate of CCR5 point mutations expressed within human HEK-293 cells. Loss of MAb reactivity by immunofluorescence can be directly attributed to specific amino acid changes in the binding epitopes. The identified residues are superimposed here on a structural model of CCR5, highlighting the extracellular loops where the epitopes are located.



**Figure 2.** Residue E172 of CCR5 was mutated to nineteen other codons, verified to express on the cell surface, and tested for binding of the MAbs 2D7 and 45523. None of the mutants, even those structurally similar to E (wt) such as Q and D, permitted 45523 binding, while only a Q substitution was able to support 2D7 binding.

## Technical Description

Shotgun Mutagenesis was used to map the binding epitopes on the chemokine receptor CCR5 for two commercially available MAbs, 2D7 and 45523. A library of eukaryotic expression plasmids coding for point mutations along the entire 1 kb CCR5 gene was prepared, and each clone was expressed in HEK-293 cells. Epitope tags at the N-terminus and C-terminus of the CCR5 construct allowed confirmation of surface expression and full-length translation of each clone. The effect of each mutation on 2D7 and 45523 MAb recognition was evaluated by immunofluorescence (**Figure 1**). The binding epitopes of each MAb were thus mapped along the entire length of the CCR5 molecule. One residue, E172, appeared critical for receptor recognition by both antibodies. To identify the atomic requirements for binding at this residue, E172 was mutated to nearly all of the other possible amino acids in order to identify acceptable and non-acceptable side-chains at that position. Using our high-throughput expression and analysis technology, each mutant was evaluated for MAb binding, surface expression, and full-length translation. Comparison of the side-chains that could or could not support 2D7 and 45523 binding permitted atomic-level structural requirements for each antibody to be identified (**Figure 2**). Shotgun Mutagenesis thus makes it possible to not only identify *which* amino acid positions are important, but also to formulate atomic-level structural hypotheses about *how* these residues contribute to receptor 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:

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