Although most drugs act via the binding site for the endogenous agonist (orthosteric site), many GPCRs possess allosteric sites that modulate receptor activity; targeting such sites can potentially lead to greater subtype selectivity for GPCRs. However, a number of fundamental issues remain unresolved with respect to mechanisms underlying GPCR activation and the basis of drug selectivity, be it orthosteric or allosteric; many of these issues relate to limitations in structural analysis. Recently, we have utilized structurally diverse allosteric modulators of the M_2 muscarinic acetylcholine receptor (mAChR), a prototypical Family A GPCR, to probe the role of the receptor’s second extracellular (E2) loop in the binding of both orthosteric and allosteric ligands. Guided by mutagenesis experiments, we developed a homology model that suggested a “hinge-like” opening of the E2 loop, prior to modulator docking and “capping” of the orthosteric site entrance. The model also predicted novel cysteine substitutions that should “lock” the E2 loop via disulfide bond formation, and significantly inhibit the binding of allosteric and orthosteric ligands. This prediction was validated experimentally using binding, kinetic and functional assays. The results argue for a dynamic and flexible role of the E2 loop in the binding of both allosteric and orthosteric GPCR ligands.