

Profile of Brian K. Kobilka and Robert J. Lefkowitz, 2012 Nobel Laureates in Chemistry

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Those of us that had been hammering away in the field for 40 years or more cannot deny the excitement in learning that Brian Kobilka, a former postdoctoral fellow of Robert Lefkowitz, in collaboration with Roger Sunahara, had determined the structure of the agonist-bound β_2 -adrenergic receptor-Gs protein complex (1). To borrow a phrase from Henry Bourne, this is “the Big Enchilada” for the G protein-coupled receptor (GPCR)/G protein field. This work followed just five years after Kobilka and Schertler’s determination of the crystal structure of the β_2 -adrenergic receptor (β_2 AR) bound to the inverse agonist carazolol (2), and over two and a half decades after the reports by Robert Lefkowitz’s group of the purification, cloning, and sequencing of the mammalian β_2 AR (3, 4). These remarkable achievements and others discussed below led to the award of the 2012 Nobel Prize in Chemistry to Kobilka and Lefkowitz. This was another big win for GPCRs, G proteins, and second messengers, a field that has already been richly rewarded, and justifiably so, for the role they play in

hormone and neurotransmitter signaling and pharmacology.

This story began over three decades ago when Bob tackled the extremely difficult job of identifying, purifying, and cloning the β_2 AR, an intrinsic membrane protein present at extremely low levels. Success evolved from the groups’ development of radiolabeled antagonists (after realizing the pitfalls of using the rapidly oxidized catecholamines), the alprenolol affinity column, and identification of batches of digitonin that would preserve activity over the 100,000-fold or so purification required. Another key to Bob’s success was his ability to attract a long list of talented postdoctoral fellows (too many to list, but notably Kobilka, Limbird, Strader, Benovic, Cerione, Lohse, Bouvier, Dohlman, Luttrell, and his longtime colleague Marc Caron). Once the task of purifying the β_2 AR was accomplished, it was delivered to Richard Dixon, Catherine Strader, and Irving Sigal at Merck, who determined a partial peptide sequence and were able to find, remarkably and luckily enough, a genomic intronless clone (3). The sequence

of the mammalian β_2 AR, along with that of the turkey β_2 AR reported by Elliot Ross’s group and the muscarinic acetylcholine receptor by Numa and colleague’s group (5), revealed the stunning similarity of the overall seven transmembrane helices topology to rhodopsin previously sequenced by Paul Hargrave et al. (6). These results linked the parallel studies of rhodopsin pioneered by Hermann Kuhn and colleagues (7), Jeremy Nathans and D. S. Hogness (8), Lubert Stryer, Krzysztof Palczewski, and many others, and suggested all GPCRs might share this homology.

Beyond the β_2 AR structure, the Lefkowitz group played a major role in establishing β_2 AR regulation as the paradigm for the GPCR field. Important areas pioneered by the group were in establishing the ternary complex model (9), and characterization of the desensitization of the β_2 AR, first demonstrating its phosphorylation in response to agonist stimulation. Interestingly, these findings were greatly aided by use of the S49 lymphoma somatic cell mutants, the cyc^- -lacking Gs, and the kin^- -lacking PKA, isolated by Henry Bourne’s group. These cell lines were used to demonstrate that desensitization of the β_2 AR proceeded through G protein-dependent and independent pathways (10–12). Building on this finding led to another landmark achievement of Bob’s group, the purification of one of the β_2 AR GPCR kinases (termed β ARK or β -adrenergic receptor kinase originally and later GRK2) from the kin^- cell line (13). Later, the group was able to show that arrestin was required for desensitization of the GRK-phosphorylated β_2 AR by binding to and completely uncoupling β_2 AR stimulation of Gs. After the discovery of the role of the G-protein $\beta\gamma$ subunits in augmenting receptor phosphorylation by GRKs (14, 15), Bob Lefkowitz and John Tesmer reported the crystal structure of GRK2 in a complex with the G-protein $\beta\gamma$ subunit (16). More recently, the Lefkowitz group was first to establish the novel concept that arrestins not only work through uncoupling and desensitizing the receptor, but also work as positive signaling proteins, scaffolding proteins such



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Brian K. Kobilka.



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as MAP kinases (17), Src (18), and many other important regulatory proteins (19).

Brian Kobilka, after leaving his postdoctoral fellowship with Lefkowitz, quickly established his own reputation in the field by achieving the first animal knockouts of the β 2AR and other adrenergic receptors, even as he was quietly and doggedly pursuing his real love of getting to the structure and molecular dynamics of the β 2AR. Brian admitted this to me during a visit at Stanford some 20 years or so back, although it was readily apparent because a good bit of our conversing was done as Brian was running in and out of his laboratory purifying a fluorescently labeled receptor. It is important to understand that at the time, Brian's goal of getting crystals and the structure of the β 2AR was one shared by innumerable groups throughout the world working on their favorite receptors. The field had the rhodopsin crystal structure as the prototype, but the crystal structure of a hormone- or neurotransmitter-regulated GPCR was elusive for the very reasons given above. Through novel and creative approaches involving a long, exhausting series of dead ends, Brian obtained workable crystals that led to the first structure of an inverse agonist-bound β 2AR with Schertler and colleagues (2). Soon after, Kobilka and colleagues and Stevens and colleagues reported a higher resolution structure based on using a fusion protein of T4-lysozyme inserted into the third intracellular loop (20, 21). These breakthroughs were trumped by Brian's determination of the structure of the agonist-bound β 2AR in complex with Gs. This required even more ingenious engineering, notably use of llama antibodies (coined nanobodies) to stabilize the activated receptor first (22) and later the

β 2AR/Gs complex (1). This work established the receptor/Gs contact sites, demonstrated the dramatic movements of the fifth and sixth transmembrane segments relative to the inactive structure, and revealed an unexpected major conformational movement of the non-Ras helical component of Gs. Brian's work has been followed by a streak of GPCR crystal structures and it is clear that these collective achievements have far reaching applicability to development of new drugs and our understanding of how agonists (strong, weak,

inverse, and biased), and potentially allosterically acting ligands differentially regulate the huge family of GPCRs. The likelihood of getting structures of GPCRs with arrestin and GRKs and determining the mechanism of biased ligands signaling looks far more promising at this juncture. As is often the case, many individuals are needed to advance a scientific field resulting in an award, as can be seen from this Profile; nevertheless Bob and Brian will be great ambassadors to represent all of this work to the world.

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