

## Measurement of Structural and Dynamic Changes in G-Proteins in Signal Transduction

*G-protein-coupled receptor (GPCR) activation of cognate G-proteins is the first step in cellular signaling cascades which mediate vision, olfaction, taste, and the action of many hormones and neurotransmitters. Due to the relevance of GPCRs in physiology and disease states, they are the focus of numerous drug discovery efforts. More than 50% of marketed drugs (excluding antibiotics) treat a broad spectrum of diseases by targeting some 20 GPCRs. Preliminary analysis of the human genome has suggested the number of therapeutically relevant GPCRs to be close to 900. Overall, our research focuses on the development of general NMR measurement methods for detecting and quantifying structural and dynamic changes in heterotrimer G-proteins associated with receptor-stimulated guanine nucleotide exchange.*

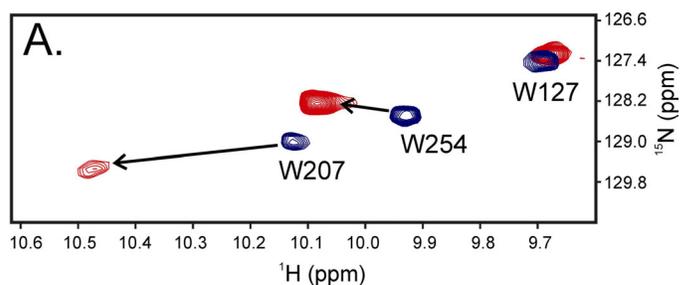
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Understanding how activated GPCR/G-protein interactions are mediated at the molecular level will provide important insights into cellular signal transduction and aid drug discovery efforts. Thus, measurement technology developed in our studies of the structural mechanism for G-protein-based signal transduction will have an impact on the biotechnology and pharmaceutical industries, as well as the structural biology program at NIST. The research has been enabled by the recent acquisition by the NMR facility at CARB/NIST of Cryoprobe technology for the 600 MHz NMR spectrometer. This technology affords approximately a four-fold increase in signal-to-noise when compared to conventional NMR probes and has enabled studies of the G-protein, which is of limited solubility and can only be concentrated to levels that are about one-half of the concentration typically necessary for NMR experiments.

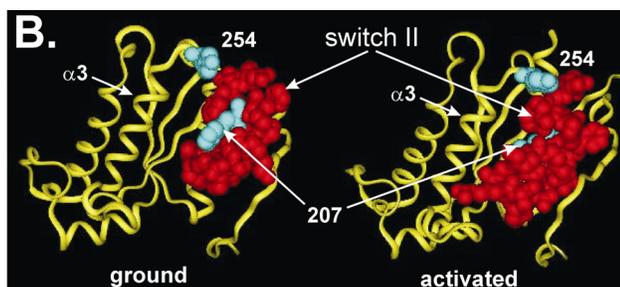
We expressed a  $G_{\alpha}$  chimera in a soluble form at high levels by using a subtilisin prodomain (proR8FKAM) fusion construct and milligram quantities of prodomain-released, full-length, isotope-labeled  $G_{\alpha}$  (ChiT) purified in a single step using an immobilized 'slow-cleaving' mutant form of subtilisin. This then allowed NMR functional studies that provided new insights into the solution structures of  $G_{\alpha}$  in various states.

We have also shown that isotope-labeled ChiT can be reconstituted with  $G_{\beta\gamma}$  subunits to form a functional heterotrimer that is amenable to structural analysis by high-resolution NMR. This latter work revealed that  $G_{\beta\gamma}$  binding to ChiT induces structural changes in the guanine nucleotide binding and carboxyl-terminal regions of ChiT, leading to a 'pre-activated' state that may facilitate interaction with activated receptor ( $R^*$ ) interactions and subsequent GDP/GTP exchange. Lastly, we have applied high-resolution NMR to begin to probe the structural basis for the propagation of signals from  $R^*$  to the G-protein, with the specific goal of developing more robust models for the structural changes in  $G_{\alpha}$  that accompany the signal transfer process. Specifically, NMR methods have been used to track the complete cycle of guanine nucleotide exchange in  $^{15}\text{N}$ -ChiT-reconstituted heterotrimer that is triggered by light-activated rhodopsin.

Structural changes revealed at atomic resolution by new NMR methods have provided a framework for industry to undertake a more rational approach for the design and development of drugs for G-coupled protein receptors.



*Expansion of the tryptophan (Trp) indole resonance region of the  $^{15}\text{N}$ -HSQC (isotopically labeled protein) spectra of GDP/ $\text{Mg}^{2+}$  (blue) and GDP- $\text{AlF}_4^-/\text{Mg}^{2+}$  (red) bound ChiT. Changes in the chemical shifts for the assigned  $^1\text{H}$ ,  $^{15}\text{N}$  cross peaks of the three Trp indoles are indicated by arrows.*



**(B)** Conformational differences in switch II between the GDP/Mg<sup>2+</sup> bound (left, PDB code 1TAG) and activated GDP-AlF<sub>4</sub><sup>-</sup>/Mg<sup>2+</sup> bound (right, PDB code 1TAD) forms of G<sub>α</sub> as observed in the crystal structures at 1.8 Å (0.18 nm) and 1.7 Å (0.17 nm), respectively. The backbones are shown in yellow ribbon, with the switch II regions in red and shown in CPK color scheme to highlight the conformational changes this region undergoes as a result of guanine nucleotide exchange. Trp residues 207 and 254 are in light blue and shown in CPK.

**\*Disclaimer:**

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**Publications**

N. G. Abdulaev, C. Zhang, A. Dinh, T. Ngo, P. N. Bryan, D. M. Brabazon, J. P. Marino, and K. D. Ridge, "Bacterial expression and one-step purification of an isotope-labeled heterotrimeric G-protein α-subunit," *J. Biomol. NMR* (2005) 32, 31-40

N. G. Abdulaev, T. Ngo, C. Zhang, A. Dinh, D. M. Brabazon, K. D. Ridge, and J. P. Marino (2005) "Heterotrimeric G-protein α-subunit adopts a 'pre-activated' conformation when associated with βγ-subunits," *J. Biol. Chem.* (2005), 280, 38071-38080.