Rowan University Department of Chemistry and Biochemistry Seminar Series

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The Functional Interplay Between Lipids, Channels, Receptors, and Biological Assemblies as Viewed by Solid-state NMR and Biophysical Techniques

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Link to Talk: https://rowan.zoom.us/j/86499691529?pwd=dW0yMmhHb1VaUjVva3hSUHptUW9EZz09

Abstract

The structures and mechanisms of membrane proteins (MPs) and lipid bilayers are irresolvable via traditional structural biology. These systems are large, complex, and highly insoluble. They lack long-rage order, and they are dynamic on multiple timescales. Solid-state NMR (SSNMR) is adept at exploring native structure-activity. The Wylie group studies MPs and functional amyloids incorporated into megadalton complexes using SSNMR. We study MPs and their functional lipids in proteoliposomes and cellular envelopes. Our work focuses on inward-rectifier K^+ (Kir) channels, G protein-coupled receptors (GPCRs), and mammalian amyloids found in the reproductive tract.

Kir channels. Kir channels conduct inward K^+ current. These channels regulate the resting membrane potential in excitable cells. Thus, they are implicated in heart disease, psychiatric disorders, and even alcoholism. We structurally characterized KirBac1.1, a bacterial channel with structural homology to mammalian channels. SSNMR revealed how lipids control the activity and conformational states of KirBac1.1. We determined the locus and mechanism of channel activation by anionic lipids and identified the coincident structural changes. We used all the experimental restraints to solve the full-length structure of KirBac1.1. The effort to solve the structure led to the finding of a new set of water-edited restraints that can be used to solve the structure of KirBac1.1 and any other protein or amyloid. We then solved the structure of the KirBac1.1-cholesterol complex. This structure uncovered a conserved Kir channel cholesterol recognition site. This cleft is a novel variation of the Cholesterol Binding Motif (CCM). It is occupied by a persistent cholesterol dimer. This structure further discerns how cholesterol efficiently prevents anionic lipids from activating the channel. We further found KirBac1.1, and potentially other membranes proteins, strongly influence membrane dynamic structure, which has clear implications for the lipid microdomain hypothesis. This work motivates emergent work on the G protein-coupled Kir channels GIRK1 and GIRK2 in our laboratory.

GPCRs. CCR3 is a C-C motif chemokine GPCR expressed by eosinophils and basophils. It transduces signals stimulated by the C-C motif chemokine primary messengers 5, 11, 24, 26, and 28. CCR3 is implicated in cancer metastasis, inflammatory conditions, and the COVID-19 cytokine storm. We developed codon harmonization techniques for heterologous production of CCR3 (and eukaryotic Kir channels), obtaining ~4 mg of functional GPCR per liter of ^{15}N,^{13}C-enriched minimal media. This allows us to efficiently investigate CCR3 function *in vitro*. Cholesterol influences GPCR-ligand interactions and receptor multimerization states. We discovered the affinity between CCR3 and the CCL11 chemokine is cholesterol dose dependent. This correlates to a dose-dependent increase in the GTPase activity of CCR3-activated $G\alpha_i3$. This work is validated by both production-quality and coarse-grain MD simulations, revealing key cholesterol binding motifs along the proposed signal transduction pathway. Preliminary SSNMR investigation of these functional mechanisms appears to confirm these results. We are also actively pursuing CCR10 and a family of promiscuous chemokines shared by the two receptors.