The Renal Outer Medullary Potassium (ROMK) channel is an important player in the kidney's ion transport system. It's like a gate that helps maintain the right balance of electrolytes in our bodies. But when ROMK malfunctions due to certain mutations, it can lead to antenatal Bartter syndrome and even affect blood pressure in humans. A special form of ROMK, called ROMK2 can be present not only in the plasma membrane or endoplasmic reticulum but also in mitochondria as a part of called mitoK(ATP) channel responsible for cytoprotection during ischemia/reperfusion events.

In our recent research, we made an exciting discovery. We found that ROMK2 forms protein complexes with two lipid kinases called acylglycerol kinase (AGK) and diacylglycerol kinase ε (DGKE). These partnerships occur within different compartments of the cell, like mitochondria and the endoplasmic reticulum.

The activity of many potassium channels, including ROMK in the outer membrane of cells, is regulated by a lipid called phosphatidylinositol 4,5-bisphosphate (PIP2). But mitochondria and the endoplasmic reticulum don't have much PIP2. However, we discovered that the products of enzymatic activity of AGK and DGKE, which are lysophosphatidic acid (LPA) and phosphatidic acid (PA) respectively, actually stimulate the activity of ROMK2 in lab-created lipid environments. This suggests that localized lipid synthesis by these lipid kinases might have a hand in controlling ROMK2 activity within these compartments.

So, in this grant proposal, we aim to dive deeper into these interactions between ROMK2, AGK, and DGKE. First, we want to understand the details of how they interact on a molecular level. We'll use special techniques to identify the specific parts of the proteins responsible for this interaction. We'll also analyze the structure of these protein complexes using cryo-electron microscopy.

Next, we want to know if these interactions affect the localization and activity of ROMK2 in living cells. We'll use renal proximal tubular epithelial cells, which have high levels of ROMK expression. By knocking out AGK and DGKE in these cells, we can see how ROMK behaves without its lipid kinase partners. We'll look at where ROMK ends up in the cells and measure potassium levels to get a better understanding of how these interactions affect its function.

Lastly, we'll investigate the impact of ROMK-lipid kinase interactions on the function of renal epithelia (the cells that line the kidney tubules). We'll use advanced techniques to study ion and water movement across these cells and see how ROMK interaction with lipid kinases impacts the survival of the cells during ischemia/reperfusion events. By manipulating ROMK activity and observing the effects on ion transport, we can gain insights into its role in maintaining proper kidney function.

Ultimately, our goal is to uncover important mechanisms that regulate ROMK channels. By understanding the interplay between ROMK2 and lipid kinases, we hope to advance our knowledge of kidney physiology. Additionally, this research may lead to the identification of new targets for treating conditions related to potassium imbalances, electrolyte disturbances, and ischemia/reperfusion events, providing potential avenues for developing future therapies against e.g. acute kidney injury.