International Doctorate in Structural Biology

Afolayan, Love Elizabeth

CHARACTERIZATION OF LIGAND BINDING TO A MEMBRANE PROTEIN BY ON-CELL NMR SPECTROSCOPY

Supervisor: Dr. Enrico Luchinat
Co-Supervisors: Prof. Lucia Banci and Prof. Roberta Pierattelli

Abstract

Ligand-observed NMR experiments monitor small-molecule ligands as they interact with biological targets. Because ligands are typically low in molecular weight with slow transverse relaxation rates, they produce sharp and well-resolved NMR signals. In contrast, target-observed approaches often suffer from severe signal loss when the target interacts with large cellular components, leading to extreme line broadening.

In this study, we applied ligand-observed NMR strategies to investigate ligand binding to the membrane protein carbonic anhydrase IX (CA IX) in intact human cells. Using an on-cell NMR approach helps overcome the challenges associated with the isolation and purification of membrane proteins. As a transmembrane protein tightly associated with the plasma membrane, CA IX is expected to experience restricted molecular tumbling, resulting in extreme line broadening. Indeed, ^{1}H-^{15}N NMR experiments revealed that CA IX is NMR-invisible in both intact cells and in cell lysates. Furthermore, no detectable signals corresponding to ligand–target complexes were observed upon treatment with fluorinated inhibitors of the carbonic anhydrase family.

Ongoing efforts to overcome these limitations include solid-state NMR studies on CA IX–enriched plasma membrane (plasma membrane vesicles) preparations and Saturation Transfer Difference (STD) NMR experiments in cells overexpressing CA IX. Preliminary results demonstrate the successful production of CA IX–enriched giant plasma membrane vesicles (GPMVs) at concentrations suitable for solid-state NMR analysis. In addition, STD experiments have detected ligand–target interactions for a moderate-affinity CA IX inhibitor, supporting the feasibility of this approach.