# Development of an *In Situ* G Protein-Coupled Receptor Fragment Molecule Screening Approach with High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance

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Over 4,000 proteins not targeted by current drugs have been identified as potential therapeutic targets, many of which are G protein-coupled receptors (GPCRs). Conventional drug screening methods rely on labeled ligands that are expensive or not commercially available, limiting which proteins can be studied. Additionally, these methods overlook lower affinity molecules that provide leads for ultimately developing drug-like compounds.

This study demonstrated a proof-of-concept application of high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR) spectroscopy for screening for small molecules that bind the human $A_{2A}$ adenosine receptor ($A_{2A}$AR), a GCPR target for Parkinson's disease and several cancers. Researchers developed a novel workflow to prepare plasma membranes enriched with $A_{2A}$AR without the need for detergent purification, which is often required in conventional experiments. This improved the sensitivity of NMR experiments while maintaining a native-like cellular environment of the plasma membrane. Researchers then used known investigational molecules such as caffeine to validate their approach before applying their method to identify novel chemical scaffolds that interact with the receptor and serve as starting points for new drug discovery.

Beyond GCPRs, HRMAS NMR could be applied to lipid-based nanoparticles, biodegradable polymeric nanoparticles, protein-excipient interactions, and antigen-adjuvant binding in drug formulations. Additionally, the method applied in this study should be readily adapted for GPCRs and other membrane proteins expressed in mammalian cells and other cell systems, underscoring its broad applicability.

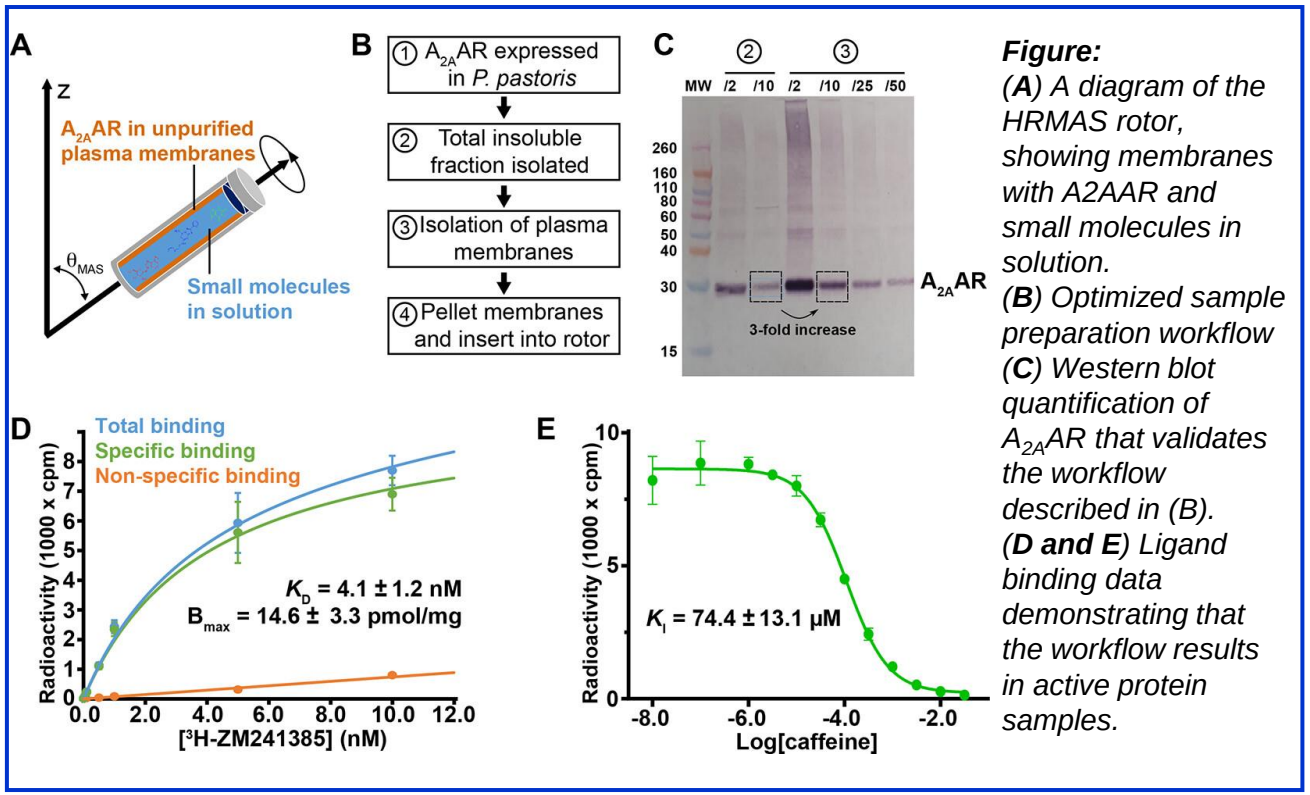


***Figure:***
*(**A**) A diagram of the HRMAS rotor, showing membranes with A2AAR and small molecules in solution.*
*(**B**) Optimized sample preparation workflow*
*(**C**) Western blot quantification of $A_{2A}$AR that validates the workflow described in (B).*
*(**D and E**) Ligand binding data demonstrating that the workflow results in active protein samples.*

**Facilities and instrumentation used:** AMRIS Facility; 800 MHz/63 mm Bruker Avance III NMR spectrometer, 4mm HRMAS HCND probe.

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