

# Cell-Free Protein Synthesis and In-Cell NMR Platforms at the Swedish NMR Centre

Ashish A. Kawale, Anders Bay Nord, Weixiao Yuan Wahlgren, B. Göran Karlsson\*

Swedish NMR Centre, University of Gothenburg, Sweden

Understanding biomolecular structures, physicochemical properties, and their implications for functions necessitates the production of high-quality biomolecules followed by characterization in a near-native environment. This poster describes the available capabilities for Cell-Free Protein Synthesis and In-Cell NMR platforms at the Swedish NMR Centre at the University of Gothenburg.

Cell-Free Protein Synthesis (CFPS) has emerged as a powerful technique for on-demand production of challenging biomolecules by utilizing cellular transcriptional and translational activities within a test tube. CFPS offers robustness and flexibility, holding great potential to address shortcomings in existing *in vivo* biotherapeutic production methods. The CFPS platform at the Swedish NMR Centre plays an integral role in Protein Production Sweden (PPS), a national-level research infrastructure dedicated to generating superior-quality protein samples. The platform provides a versatile toolbox for rapid and cost-effective *in vitro* protein synthesis, capable of producing diverse types of protein samples (e.g., soluble, membrane-bound, toxic) from a plasmid template using isolated translation machinery from bacterial (*E. coli*) extracts. NMR-optimized isotopic labeling for proteins through the CFPS protocol with subsequent NMR data acquisition and analysis is also available. Current efforts are focused on improving the in-house developed system and extending CFPS applications through optimized protocols using yeast and mammalian extracts. The methodology is available to local and external researchers upon request through PPS projects.

Similarly, In-cell NMR offers an amazing tool to study biomolecules such as proteins and nucleic acids at an atomic level in their natural environments, i.e., living cells. This enables researchers to characterize the biomolecular structure, functional properties, intermolecular interactions, and dynamics in real-time under physiological conditions, offering a wide range of biotechnological applications. Equipped with the InsightCell Bioreactor from Bruker, our In-cell NMR platform can perform real-time NMR studies on living cells supplemented with appropriate nutrients, substrates, and drugs, keeping cells alive during measurements. We offer access to user support and instrumentation upon request.