1. Title of Case Study: Characterising large protein complexes by >60 kHz magic angle spinning NMR

2. Grant Reference Number: Contract: PR140003

3. One sentence summary: High-field solid-state NMR provides an atomic resolution view of structural and dynamic aspects of protein-protein interactions in large protein complexes that are inaccessible to other high-resolution techniques.

4. One paragraph summary: Lewandowski and co-workers have developed and applied a series of new methods that take advantage of high magnetic field and fast, up to 100 kHz, magic angle spinning. This has led to atomic resolution characterisation of structures, dynamics and interactions of proteins in large complexes that are either inaccessible or challenging for other high-resolution structural techniques. The proposed approach has enabled quantitative studies on complex systems where the observed component was present in minute, even as small as 10 μg, quantities. The ability to perform quantitative measurements on such small amounts of sample paves a way to understanding mechanistic details for a myriad of biological processes that rely on interactions between different biomolecules to perform their function. The developed methods were applied to a >300 kDa precipitated complex of protein GB1 with full length antibody, yielding information concerning the conformation and dynamics of GB1 in the complex and its interactions.

5. Key outputs in bullet points:

- *Demonstration of a new approach that provides atomic resolution information on structures, dynamics and interactions in large biomolecular complexes that are inaccessible to other high-resolution techniques using samples where the observed component is present in very small (<50 μg) quantities*
- *First site-specific measurements of ps-ms dynamics in a >300 kDa complex in the solid state*
- *Demonstration of solvent Paramagnetic Relaxation Enhancements as a tool to characterise intermolecular interfaces in large biomolecular complexes in the solid state*
- *Contribution to the development of a unique 0.81 mm 100 kHz magic angle spinning probe and demonstration of its utility for studies of biomolecules*
- *Training in solid state NMR of two PhD students, Dr Jonathan Lamley (currently employed in the aerospace industry) and Carl Öster (currently employed by Medicines Discovery Catapult) and two postdoctoral researchers: Dr Angelo Gallo (currently an ERC-funded fellow) and Dr Simone Kosol (currently a BBSRC-funded fellow)*
- *New collaboration with Prof Stephan Grzesiek (U. of Basel, Switzerland), Dr Tobias Madl (U. of Graz, Austria), Prof David Roper (U. of Warwick) on studies of large protein complexes*

6. Main body text

After water, proteins constitute the largest fraction of cellular content so it is not surprising that a large number of processes in cells are performed by proteins that interact with other proteins and molecules. In fact, most genes critical for cell survival encode protein complexes. Knowledge of how proteins interact and how they change at the molecular level as a result of these interactions is often essential for understanding mechanisms of their function and malfunction. In turn, such understanding is key to figuring out how to fix the complex protein machineries when they go wrong, e.g., in diseases, or how to bend them to our will, e.g., to produce new bioactive compounds

using synthetic biology approaches or to develop practical solutions for tackling renewable energy challenges. However, large protein complexes with multiple components present very serious challenges to existing analytical methods, e.g. due to their size, solubility or inability to form crystals of a quality suitable for structure determination by X-ray crystallography. For many systems facing such challenges, solid-state NMR could potentially provide atomic resolution information in analogy to solution NMR but without the same limitations regarding size and solubility. The aim of this work is to develop a general approach for obtaining molecular level structural and dynamic information in large protein complexes using fast magic angle spinning solid state NMR at high magnetic field. In the first instance, we have demonstrated on an insoluble precipitated > 300 kDa complex of protein GB1 with immunoglobulin G (IgG), which is not suitable either for solution NMR or X-ray crystallography, that the combination of ^{1}H detection enabled by fast, 60-100 kHz, magic angle spinning and optional paramagnetic doping makes possible quantitative measurements on complex samples containing isotopically labelled GB1 in quantitates in the 50-10 μg range (Lamley, J. M. *et al.*; Lewandowski, J. R. *J. Am. Chem. Soc.* **2014**, 136, 16800). We were able to use the measured chemical shifts to describe both the interactions and overall conformation of GB1 in the complex. Subsequently, we have performed the first ever site-specific measurements of NMR relaxation to characterise dynamics in such a large complex in the solid state, and provide valuable information concerning the influence of intermolecular interactions on motions in molecules (Lamley, J. M. *et al.*; Lewandowski, J. R. *Angew. Chem.* **2015**, 54, 15374.). We have also developed an approach based on measuring solvent Paramagnetic Relaxation Enhancements (sPREs) to characterise protein-protein interactions in such large complexes (Öster, C. *et al.*; Lewandowski, J. R. *J. Am. Chem. Soc.* **2017**, 139, 12165). This new method has enabled us to detect a new specific protein-protein interface between GB1 and IgG that could not have predicted from the studies of soluble protein complexes of protein G with fragments of IgG. This result highlights the importance of working with full size constructs and informs us of how using fragments can yield incomplete information. It is also important to point out that this project has provided us with an opportunity to aid development, in collaboration with Prof. Ago Samoson (Technical University of Tallinn, Estonia), of a unique 0.81mm probe capable of magic angle spinning at 100 kHz. The collaboration has helped to customise this instrument to be suitable for applications on biological systems and has enabled us to perform sPRE measurements based on recording ^{1}H relaxation, which are impossible with the previously available equipment (Öster, C. *et al.*; Lewandowski, J. R. *J. Am. Chem. Soc.* **2017**, 139, 12165.). Currently, we are in the process of applying similar methods to a range of protein complexes involved in the biosynthesis of important antibiotics or protein complexes involving membrane proteins.

7. Names of key academics and any collaborators:

Dr Józef Lewandowski (University of Warwick); Prof Ago Samoson (Technical University of Tallinn, Estonia); Dr Tobias Madl (Univeristy of Graz, Austria); Prof Stephan Grzesiek (University of Basel, Switzerland)

8. Sources of significant sponsorship (if applicable): *ERC (Starting Grant 639907), Royal Society RG130022, EPSRC EP/L025906/1, BBSRC BB/L022761/1; Contract for the High Field Solid State Nuclear Magnetic Resonance Facility (EPSRC);*

9. Who should we contact for more information?

Dr Józef Lewandowski, j.r.lewandowski@warwick.ac.uk