Ligand screening methods employing by NMR spectroscopy have become well established techniques for the study of small molecules binding to macromolecular targets and have a role to play in the development of new pharmaceuticals and novel ligands designed as probes to investigate biochemical pathways. The methodologies available generally fall into two classes: “protein observe” where the resonances of the (isotopically labeled) macromolecule are detected or “ligand observe” where the behavior of the small molecule itself is monitored. In the absence of isotopically labeled protein, ligand observed techniques are favored and methods such as saturation transfer difference (STD) and WaterLOGSY are widely employed.

In some cases, however, techniques employing direct observation of the binding ligand can be susceptible to false negatives (high-affinity ligands may give no detectable response on binding) and false positives (non-specific binding to the macromolecule). In such cases the use of “reporter” or “spy” molecules in competition based assays may overcome these limitations whereby the response of the reporter is monitored as it becomes displaced by the ligand of interest under investigation. Herein we describe the application of reporter-based NMR screening methods to members of the so-called 2-oxoglutarate (2OG) dependent oxygenases. This family of proteins comprises Fe(II) dependent enzymes that target either small molecules or complete proteins as substrates for hydroxylation. These enzymes have many essential roles in mammalian biology including, for example, responses to oxygen levels, fatty acid metabolism and epigenetic regulation. Examples of reporter ligand methods will be described, including the use of the cofactor 2OG as a generic reporter for the enzyme family [1] and the use of solvent water itself as a reporter [2].