MIRAGE NMR Guidelines – Glycan Recognition

Guidelines for Reporting Nuclear Magnetic Resonance Data on Binding of Glycans to Receptors

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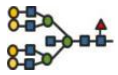
These guidelines are proposed to comprehensively describe the NMR experiments and data obtained to characterize glycan recognition by various receptors. The receptor can be a glycan binding protein (such as lectins and antibodies), a glycan binding organism (such as cells) or can have a different nature including synthetic glycan binding molecules.

	Description*
1. Sample: Glycan Binding Sample	
Glycan/receptor description	**Glycan descriptors** Origin: Natural/synthetic/glycoprotein Structural descriptors Glycan sequence, bond regio chemistry (1-2/1-4/1-6) and stereochemistry (α/β) For unnatural glycans describe: reducing-terminal modifications; artificial functional groups; isotopic labelling. Molecular Size (exact mass/average MW) **Receptor description** (Sequence, MW, modifications) 
Description of Sample	Carbohydrate: receptor, molar ratio Concentration Buffer composition Solvent Chemical Shift Reference (e.g. acetone at 2.218 ppm for ^{1}H and 33.0 ppm for ^{13}C). Stability (temperature and pH requirements)
Quality control	Quaternary structure and Oligomerization state Impurities (natural/chemicals)
2. Spectrometer and Data Processing	
Spectrometer	Magnetic field/cryoprobe y/n
Manufacturer	
Experiments	Indicate: 1D/2D/3D; Name: (^{1}H, ^{1}H-^{1}H tr-NOESY, ^{1}H-^{13}C HSQC, ^{1}H-^{15}N HSQC, STD, Waterlogsy); Specify pulse sequence: (if a pulse sequence is modified report the sequence).

	Describe specific parameters of the experiments (e.g. number of scans, spectral width, number of increments, mixing time)
Measuring conditions	Temperature
Data processing	Window functions (LB, GB), zero filling, baseline corrections
3. NMR parameters	
Glycan chemical shifts (free/bound states)	δ (^{1}H), δ (^{13}C) list reported y/n
Receptor chemical shifts	δ (^{1}H), δ (^{13}C), δ (^{15}N) protein, list reported y/n Chemical shift perturbation mapping y/n
Intensity attenuation	y/n, Describe signals with line broadening upon interaction
Glycan Js (free/bound states)	y/n, J (^{1}H-^{1}H, ^{1}H-^{13}C) list reported y/n, J order
Glycan NOEs (free/bound states)	y/n, NOE list reported y/n, intermolecular NOEs y/n
Glycan relaxation times (free/bound states)	y/n, T1, T2 values reported y/n
Glycan RDCs, PCSs, PREs	y/n, RDC (^{1}H-^{1}H, ^{1}H-^{13}C) values reported y/n
Saturation Transfer Difference Effect (STD)	STD intensities y/n, STD amplification factors y/n
4. NMR Data Presentation	
Data presentation	Figures and tables, epitope mapping
5. Interpretation and Conclusion from NMR data	
Data interpretation	
Conclusions	

*Only items relevant to NMR experiments need be included in this document which may be cited in manuscripts as a Supplementary Table. Descriptions and references cited here should complement rather than duplicate the content of the Materials and Methods.