Uniformly ^{13}C-labeled carbohydrates for probing carbohydrate-protein interactions by NMR spectroscopy

Gustav Nestor,[a,b] and Angela M. Gronenborn,[a]

[a] Dept. of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[b] Dept. of Molecular Sciences, Swed. Univ. of Agricultural Sciences (SLU), Uppsala, Sweden

Carbohydrates used in structural studies by NMR are rarely ^{13}C-labeled, despite severe resonance overlap in their ^{1}H NMR spectra. This is caused by the lack of easily accessible isotope-labeled material, preventing exploitation of the ^{13}C spectral dispersion in 3D or higher order NMR experiments. In this presentation, we demonstrate the power of using uniformly ^{13}C-labeled mannosides to delineate the carbohydrate-protein interface by tailored isotope-filtered experiments.[1] As model system we selected the complex of mannosides bound to the antiviral protein cyanovirin-N (CV-N). Using different isotope labels on the glycan and the protein, our approach provides a versatile means for simultaneously mapping binding interfaces on both a carbohydrate and its protein binding partner. We also identified carbohydrate hydroxyl protons that form hydrogen bonds with CV-N backbone carbonyl oxygens.[2] Their resonances, surprisingly, were observable in the room-temperature spectra of the complex and were assigned via scalar couplings from the adjacent sugar ring protons. Intra- and intermolecular NOEs involving these hydroxyl protons permitted the determination of their orientation and hydrogen-bonding patterns. Overall, our results emphasize the general applicability of our novel approach for characterizing hydrogen bonding in carbohydrate-protein interactions.

[1] G. Nestor et al. *J. Am. Chem. Soc.* **2017**, *139*, 6210-6216.

[2] G. Nestor et al. *J. Am. Chem. Soc.* **2018**, *140*, 339-345.