Analysis of protein-ligand hydrogen bonding patterns in galectin-3 by neutron crystallography guides drug design

F. Manzoni,[a,b,†] R. Kumar,[a] O. Caldararu,[c] T. Schrader,[d] A. Ostermann,[e] L. Coates,[f] M.P. Blakeley,[g] E. Oksanen,[b] Ulf Ryde,[c] and **Derek T. Logan[a]**

[a]Biochemistry & Structural Biology, Dept. of Chemistry, Lund University, Sweden
[b]Instrument Division, European Spallation Source ERIC, Lund, Sweden
[c]Theoretical Chemistry, Dept. of Chemistry, Lund University, Sweden
[d]Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ), Forschungszentrum Jülich GmbH, Garching, Germany
[e]Heinz Maier-Leibnitz Zentrum (MLZ), Technische Universität München, Germany;
[f]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA
[g]Institut Laue-Langevin, Grenoble, France
[†]Passed away on 12th March 2017

Galectin-3 is an important protein in molecular signalling events involving carbohydrate recognition, and an understanding of the hydrogen-bonding patterns in the carbohydrate binding site of its C-terminal domain (galectin-3C) is important for the development of potent new inhibitors. We have studied these patterns using neutron crystallography. We present neutron crystal structures of galectin-3C in three states: apo, in complex with the natural substrate lactose and with glycerol. These structures reveal the exquisite tailoring of the carbohydrate recognition site to recognise the hydrogen bonding patterns presented by galactose and glucose moieties in unstrained conformations. Comparison of the glycerol and lactose structures reveals the possible importance for molecular recognition of a hydrogen bond from arginine to the cyclic oxygen atom of galactose. The apo structure shows that, though water molecules occupy the positions of the most important oxygen atoms of the ligand[1], not all hydrogen bond directionality is preserved when the oxygen atoms are unconstrained by being "locked" in the ligand. These insights have implications for ongoing design of potent inhibitors of galectin-3C interactions.

I will also present briefly some of the work required to achieve these structures, e.g. successive improvement in crystal size for perdeuterated galectin-3C by development of a crystal growth protocol involving feeding the crystallisation drops[2]. The larger crystals resulted in improved data quality and reduced data collection times. Finally, I will discuss the implementation of quantum chemical methods in joint X-ray/neutron refinement of protein crystal structures[3].

1. K. Saraboji, M. Håkansson, S. Genheden, C. Diehl, J. Qvist, U. Weininger, U.J. Nilsson, H. Leffler, U. Ryde, M. Akke & Logan DT. *Biochemistry* **2012**, *51*, 296–306.

2. F. Manzoni, K. Saraboji, J. Sprenger, A.L. Noresson, U. Nilsson, H. Leffler, Z. Fisher, T. Schrader, A. Ostermann, L. Coates, M.P. Blakeley, E. Oksanen & D.T. Logan. *Acta Crystallogr. D. Biol. Crystallogr.* **2016,** *72*, 1194-1202.

3. O. Caldararu, F. Manzoni, E. Oksanen, D.T. & U. Ryde *Acta Crystallogr. D Biol. Crystallogr.* **2019,** *75*, 368–380.