Experimental report for ESRF Expt. MX-1555, proposer Clemens Grimm, University of Wuerzburg, Beamline ID23-1

Overview

Galectin-1, a prominent member of animal lectins, is overexpressed in malignant tissues and involved in numerous types of cancer.[1, 2, 3] The protein exhibits characteristic carbohydrate-recognition domains on opposite sites of the homodimeric structure and interacts selectively with β-galactosides (like lactose and *N*-Acetyllactosamine) of glycoconjugates on cell surfaces. These interactions introduce biomolecular processes as cell proliferation, apoptosis and tumor progression.

The binding constants of natural carbohydrates are low and range in the micromolar.[4, 5] This fact demonstrates the urgent need for the identification and development of highly affine and selective ligands. Our work presents a rational approach for the design of novel Galectin-1 ligands and aims at introducing binding partners as potential lead structure for therapeutical drug development.

Based on the crystal structure solved in the previous experiment (MX 1495), we have now predicted computationally a set of derivatives of ligand 1 (Fig. 1) and synthetized the 11 highest scoring hits via an azide in a Sharpless-Huisgen-Meldal "click reaction" .

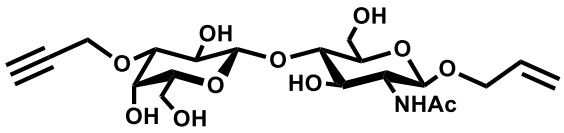

Fig. 1: Ligand 1, a N-Acetyllactosamine derivative serving as reaction partner for the Sharpless-Huisgen-Meldal "click reaction".

Crystals soaked with the two top scoring compounds NB169 and AN027 (Fig. 2) produced significant, well-defined electron density.

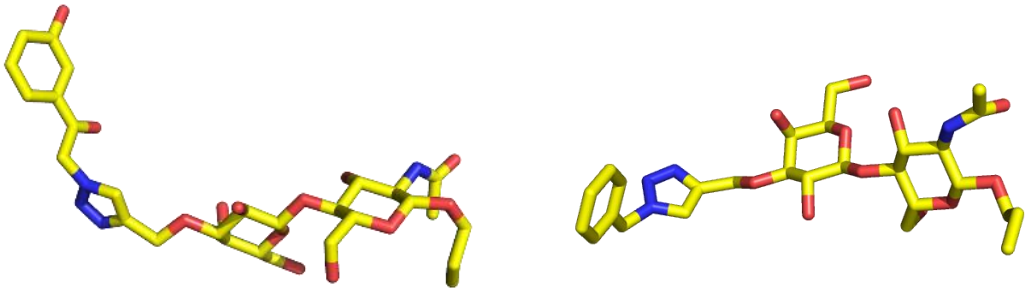

Fig. 2: Derivatives of the natural carbohydrate *N*-Acetyllacotsamine produced via the "click"-reaction. Left: compound AN027; right: compound NB169.

These results represent a significant step towards novel high-affinity Gal1 ligands with potential pharmaceutical and therapeutical significance.

Evaluation and results

During this session we have measured more than 40 datasets soaked with 11 different *N*-Acetyllactosamine derivatives synthesized via the Sharpless-Huisgen-Meldal "click reaction". All soaked crystals showed comparable diffraction to around 1.5 Å resolution. For ligands AN027 and NB169 we were able to observe significant and well-defined electron densitiy (Fig. 3, see also Table 1 for data collection and refinement statistics).

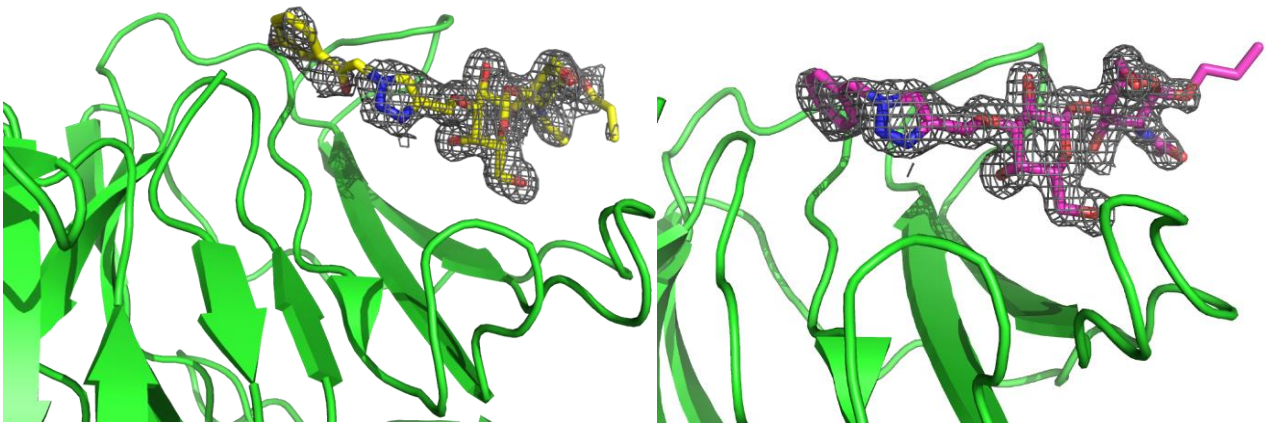

Fig. 3. Left: compound AN027 bound to Gal1. Electron density contoured on 1.0σ level; Right: compound NB169 bound to Gal1. Electron density contoured on 0.8σ level.

Table1: Data collection and refinement statistics of the NB169 soaked crystal

Data Collection	
Beamline	ID23-1
Wavelength (Å)	0.9764
Space group	P212121
Cell dimensions a, b, c (Å)	 43.3, 58.4, 111.1
No. molecules in asymmetric unit	2
Resolution (Å)	55-1.45
Rsym (%)	6.3
Mean I/σ(I)	13.44
Completeness (%)	98.6
Refinement	
R / R_{free}	0.150 / 0.187
RMS deviations Bond lengths (Å) Bond angles (°)	 0.010 1.475
Ramachandran Favoured, Allowed, Outliers [%]	 96.8, 1.9, 0.0

References

(1) Thijssen, V.; Poirier, F.; Baum, L. G.; Griffioen, A. W. *Blood* **2007**, *110*, 2819.

(2) Rabinovich, G. A.; Toscano, M. A. *Nat. Rev. Immunol.* **2009,** *9*, 338-352.

(3) Vasta, G. R. *Nat. Rev. Microbiol.* **2009,** *7*, 424-438.

(4) Camby, I.; Le Mercier, M.; Lefranc, F.; Kiss, R. *Glycobiolgy* **2006**, *16*, 137R.

(5) Lopez-Lucendo, M. F. ; Solis, D.; André, S.; Hirabayashi, J.; Kasai, K.; Kaltner, H.; Gabius, H. J.; Romero, A. *J. Mol. Biol.* **2004**, *343*, 957.

(6) Rostovsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.

(7) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.