Experimental report for ESRF Expt. MX-1495, proposer Clemens Grimm, University of Wuerzburg, Beamline ID14-4

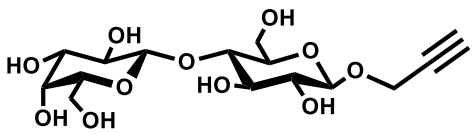
Overview

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.

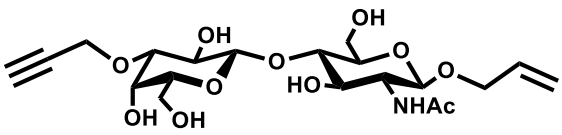
The binding constants of natural carbohydrates are low and range in the micromolar.[4, 5] This fact demonstrates the urgent need for the identification and development of highly affine and selective ligands. Our work presents a rational approach for the design of novel Galectin-1 ligands and aims at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

To realize this intent natural (lactose and *N*-Acetyllactosamine) with an artificial bioorthogonal function in variable positions of the carbohydrate structure were docked with the crystal structure of Galectin-1.[5] Promising candidates (**1** and **2**) were synthesized in our lab and co-crystallized with Galectin-1. The structure elucidation of these complexes will allow us to identify amino acids that are located adjacent to the carbohydrate binding domain. To address these amino acids and generate a highly affine binding partner the adequate functional group is inserted in a following step as an azide by the Sharpless-Huisgen-Meldal "click reaction".[6,7]

(1)



(2)



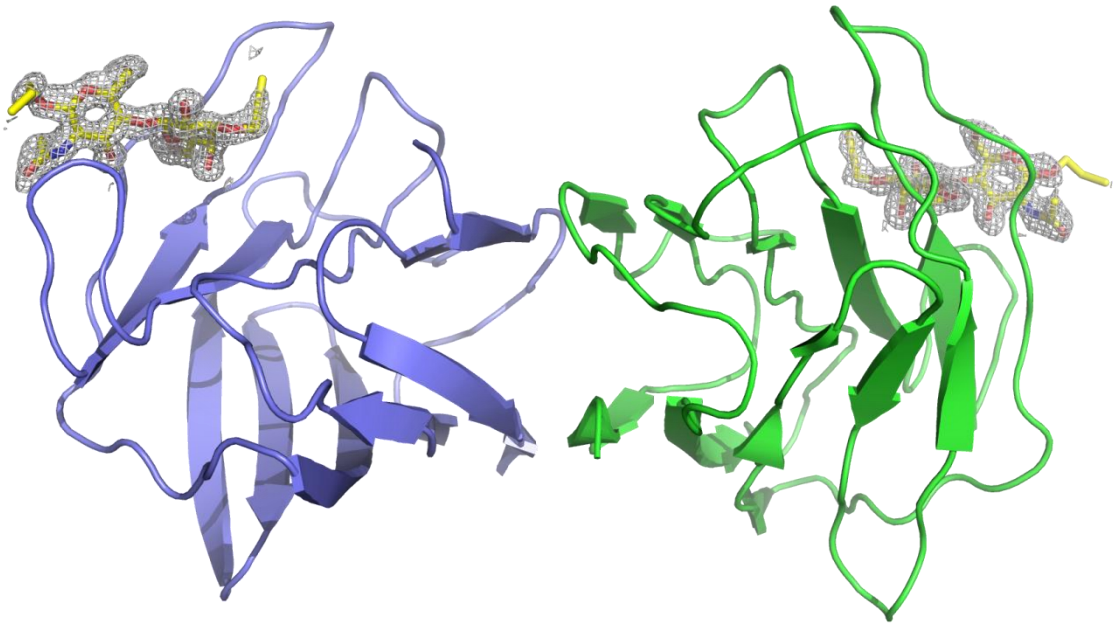
Evaluation and results

After testing a variety of differently soaked or co-crystallized crystals, we were able to collect several datasets that allowed clear identification of density for bound *N*-Acetyllactosamine as well as ligand (1) and ligand (2) (see Fig. 1 and Table 1 for data collection and refinement statistics). We are currently preparing a variety of click-reaction products to create ligands with increased affinity for Gal1.

Table1: Data collection and refinement statistics of the 6S complex crystal

Data Collection	
Beamline	ID14-4
Wavelength (Å)	0.9792
Space group	P212121
Cell dimensions a, b, c (Å)	 43.2, 58.2, 111.3
No. molecules in asymmetric unit	2
Resolution (Å)	55-1.3
Rsym (%)	9.7
Mean I/σ(I)	19.2
Completeness (%)	96.5
Refinement	
R / R_{free}	0.174 / 0.212
RMS deviations Bond lengths (Å) Bond angles (°)	 0.012 1.520
Ramachandran Favoured, Allowed, Outliers [%]	 98.3, 1.8, 0.0

Fig. 1: Ligand (2) bound to the Gal1 dimer. Model density for the ligand contoured at 0.9 σ.



References

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