BIOLOGICAL
CRYSTALLOGRAPHY

Volume 71 (2015)

Supporting information for article:

Structural bases for N-glycan processing by mannoside-phosphorylase

Simon Ladevèze, Gianluca Cioci, Pierre Roblin, Lionel Mourey, Samuel Tranier and Gabrielle Potocki-Véronèse

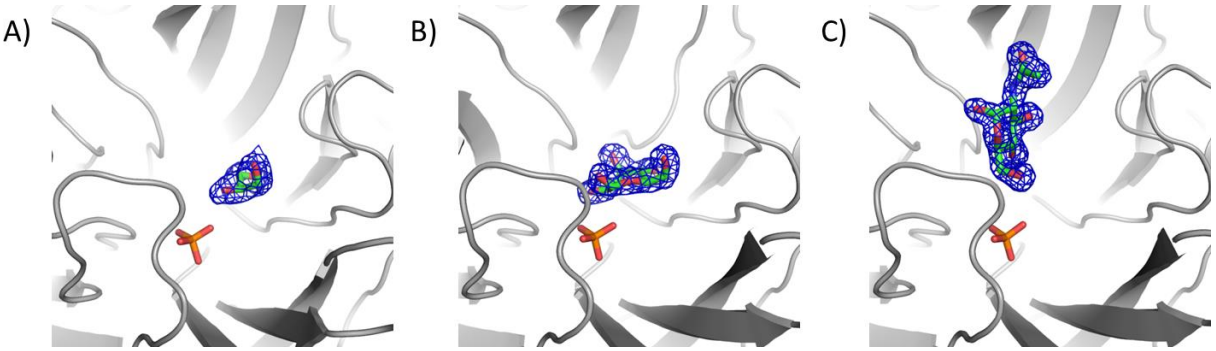

Figure S1 2Fo-Fc electron density maps contoured at 1,0 σ around the ligands. A) *apo* form, with a glycerol bound in the -1 sub-site; B) mannose bound in the -1 sub-site; C) *N*-acetylglucosamine bound in the +1 sub-site.

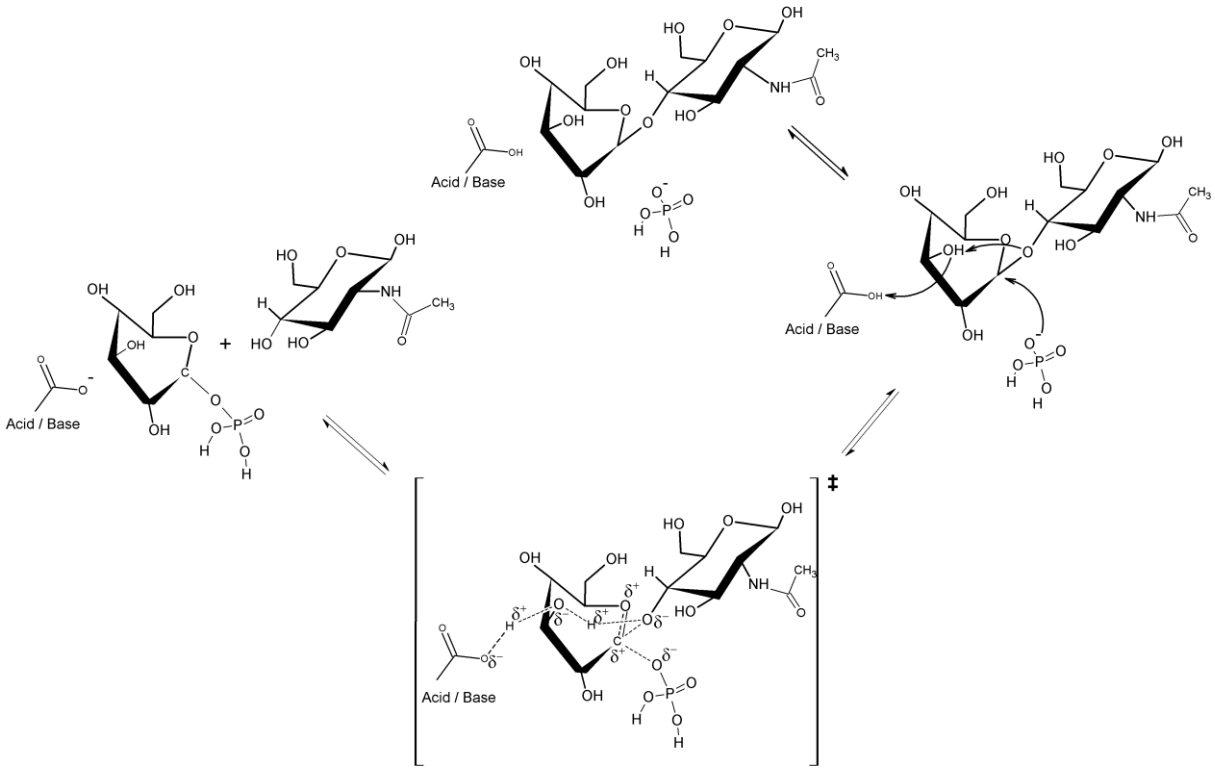

Figure S2 Proposed catalytic mechanism of β-D-Man*p*-1,4-D-Glc*p*NAc phosphorolysis by UhgbMP. This mechanism is similar to the one described by Nakae et al. for the GH130_1 *Bf*MGP protein. It involves the assistance of the C_3-OH of the mannosyl residue in the -1 sub-site to relay the proton from the catalytic D104 to the *N*-acetylglucosamine O_4.

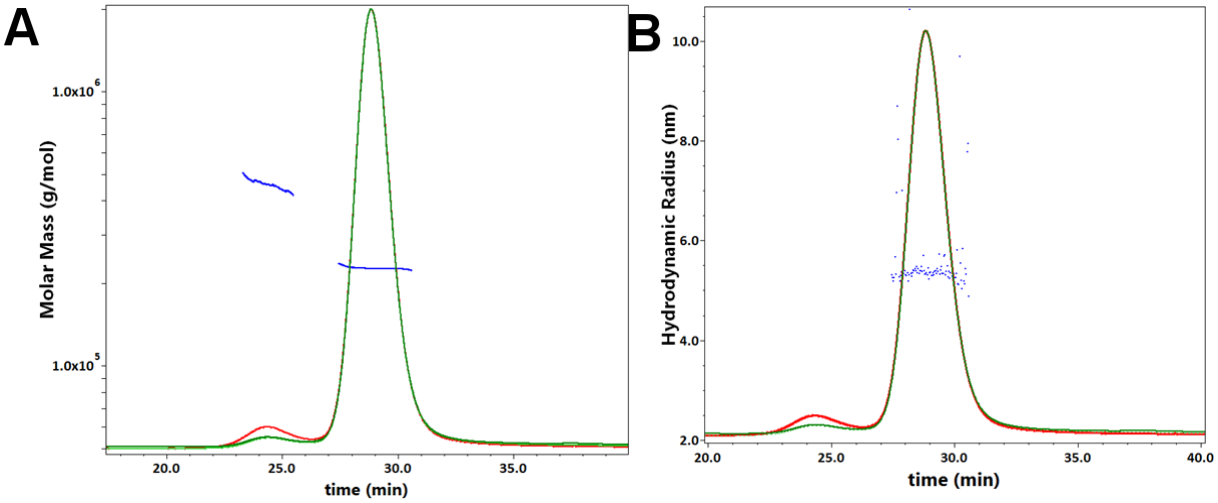

Figure S3 Size Exclusion Chromatography Multi-Angle Laser Light Scattering (SEC-MALLS) profile of Uhgb_MP. Protein apparent molecular mass was 240 kDa (n= 6.16). The main peak corresponding to the hexameric form accounted for 94% of the total mass fraction (Rh of 5.4 nm), with the presence of a small peak (3% of the total mass) that could correspond to a higher oligomeric form, possibly a dodecamer. A. Profile of the SEC-MALLS experiment displaying the light scattering signal (red curve) and the differential refractive index signal (green curve) versus elution time (min) for Uhgb_MP. The blue curve represents the molar mass ($g.mol^{-1}$) calculated across the elution peak according to SLS measurement. The average molar mass ($2.277x10^5$ ±0.025% $g.mol^{-1}$) is indicated by the dashed black line. B. Profile of the SEC-MALLS experiment showing the light scattering signal (red curve) and the differential refractive index signal (green curve) versus elution time (min) for Uhgb_MP. The blue curve represents the hydrodynamic radius (nm) calculated across the elution peak according to SLS measurement.

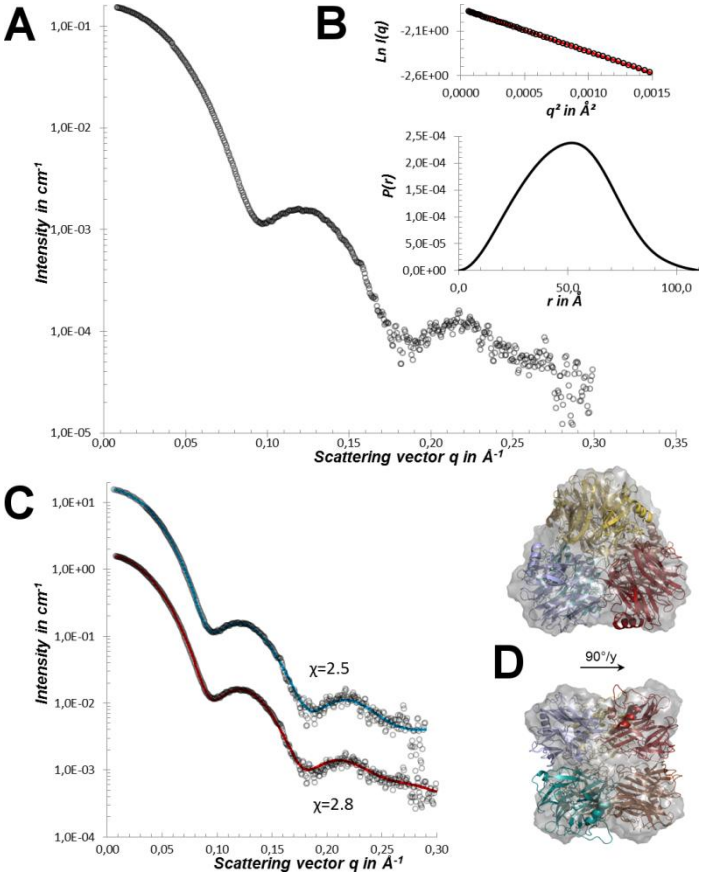

Figure S4 SAXS analysis. A. SAXS curve of Uhgb_MP in Tris-glycerol buffer supplemented with 1mM TCEP. B. Guinier fit and P(r) function. C. Fits of the ab-initio envelope (blue curve) and the crystal structure with modelled N-terms (red curve). D. Two orthogonal views of the model (shown as ribbon) superposed to the ab-initio envelop.

S1. Supplemental experimental procedures

S1.1. Uhgb_MP production and purification

The Uhgb_MP encoding gene was subcloned from pDEST17 vector (Ladevèze et al. 2013) into pET28a between NdeI and BamHI restriction sites, yielding a 6-histidine N-terminal thrombin cleavable tagged protein. Primers forward 5’ TACGCTAGCCATATGAGTATGAGTAGCAAAGTT 3’ and reverse 5’ TACGCTGGATCCTCAGATGATGCTTGTA 3’ were used to amplify the gene, using the Phusion® High-Fidelity DNA Polymerase (New England Biolabs), and to introduce NdeI and BamHI restriction

sites on both ends of the PCR product. They were subsequently digested, purified and ligated together. After *E. coli* TOP10 transformation, the construct was extracted and checked by Sanger sequencing.

To allow heterologous Uhgb_MP production, *E.coli* BL21-AI cells (Invitrogen) transformed with the Uhgb_MP::pET28a plasmid were cultured at 20°C for 24 hours in 200mL ZYM-5052 auto-induction medium (Studier 2005) supplemented with 50 µg/mL Kanamycin, inoculated at OD_{600} nm of 0.1. Next, two purification protocols were designed, protocol 1 to prepare the protein in 20 mM Tris-HCl pH 7.0, 300 mM NaCl for DSF experiments and first SAXS trials, and protocol 2, to prepare it in 20 mM potassium phosphate pH 7.0, 150 mM NaCl, for protein crystallization, SEC-MALLS and further SAXS experiments. Protocol 1: After culture, the cells were harvested and resuspended at an OD_{600} nm of 80 in lysis buffer 1 (50 mM Tris-HCl, pH 7.0, 300 mM NaCl, 10% (w/v) glycerol, 40 mM imidazole), supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche), 0.75 mg/ml lysozyme, and lysed by sonication. After centrifugation at 15,000 g for 30 min, soluble lysate was filtered through 0.2µm disposable filters (Sartorius) before loading onto a Histrap HP 1ml column equilibrated with lysis buffer 1 and connected to an ÄKTA Express (GE Healthcare) device. The column was washed with 15 volumes of affinity buffer 1 (20 mM Tris-HCl, pH 7.0, 300 mM NaCl, 10% (w/v) glycerol) supplemented with 200 mM imidazole, and the protein was then eluted in affinity buffer 1 supplemented with 500 mM imidazole. Fractions containing pure protein were identified by SDS-PAGE, pooled, and concentrated to 9–12 mg/ml. The concentrated pool was then applied onto a HiLoad 16/60 Superdex 200 (Amersham Biosciences) column and eluted using gel filtration buffer 1 (20 mM Tris-HCl pH 7.0, 300 mM NaCl). Uhgb_MP was purified to homogeneity with a yield of 70 mg/L of culture.

Protocol 2: After culture, the cells were harvested and resuspended in lysis buffer 2 (50 mM potassium phosphate pH 7.0, 150 mM NaCl, 10% (w/v) glycerol, 40 mM imidazole) supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche) and 0.75 mg/ml lysozyme to an OD_{600} nm of 80. The subsequent purification steps were the same as in Protocol 1, but affinity buffer 1 was replaced withby affinity buffer 2 (20 mM potassium phosphate pH 7.0, 150 mM NaCl, 10% (w/v) glycerol), and gel filtration buffer 1 was replaced with gel filtration buffer 2 (20 mM potassium phosphate pH 7.0, 150 mM NaCl).

S1.2. Differential Scanning Fluorimetry

Two in-house 96-well plates were designed to monitor pH stability in the range 4.5-9.0 using various common buffer species (citrate, acetate, phosphate, MES, PIPES, HEPES, TRIS, Imidazole, Bicine, and CHES) at a final concentration of 100 mM and increasing NaCl concentrations ranging 136 to 439 mM NaCl. Samples were loaded into a 96-well PCR plate (Bio-Rad) at a final volume of 11 µl. The concentration of protein in each well was 11 µM (0.4 $mg.ml^{-1}$), and 5X SYPRO Orange (Invitrogen) was added. DSF experiments were carried out using a CFX96 real-time PCR system (Bio-Rad) set to use the 480nm/500nm excitation and 560nm/580nm emission channels. The samples were heated from 20°C to 89.9°C. A single fluorescence measurement was taken every 0.3°C and each measurement lasted 6.5s. *Tm* was given by the inflection point of the curve of relative fluorescence unit (rfu) as a function of temperature (rfu=$f(T)$). The degree of thermal shift (ΔTm) was calculated as follows: $\Delta Tm = Tm(x) - Tm_0$, $Tm(x)$ being the *Tm* measured in each condition, and Tm_0 being the *Tm* of reference, measured in the purification buffer.

Ladevèze S, Tarquis L, Cecchini DA, Bercovici J, André I, Topham CM, Morel S, Laville E, Monsan P, Lombard V, Henrissat B, Potocki-Véronèse G (2013) J Biol Chem 288:32370–32383.

Studier FW (2005). Protein Expr Purif 41:207–234.