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Supporting information for article:

Structures of the transcriptional regulator BgaR, a lactose sensor

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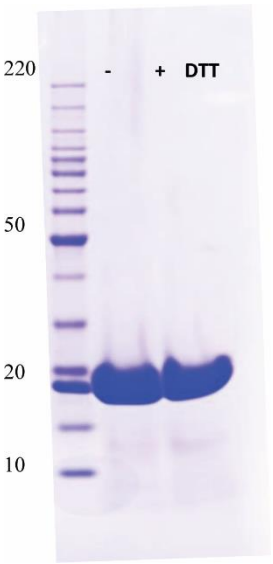


Figure S1 SDS-PAGE of purified $BgaR_{1-170}$-Thrombin-His_6. The protein was diluted to 1 mg/mL and 6 µL of BgaR was adjusted to 15 µL with 1x PBS, to which 5 µL of 4x LDS-SB (Invitrogen) and +/- 1 µL of reducing agent (2.5 M DTT) was added. The samples were heated to 95° C for 5 minutes and centrifuged briefly. 19 µL was loaded onto 4-12% NuPage gel in 1x MES buffer, 200V for 36 minutes. Stained with Coomassie Blue R250 (ethanol/ acetic acid). The left lane is molecular weight markers with the top, bottom and two dark bands labelled. The right lane was the protein with DTT added and the middle lane was the protein without DTT added to the sample buffer.

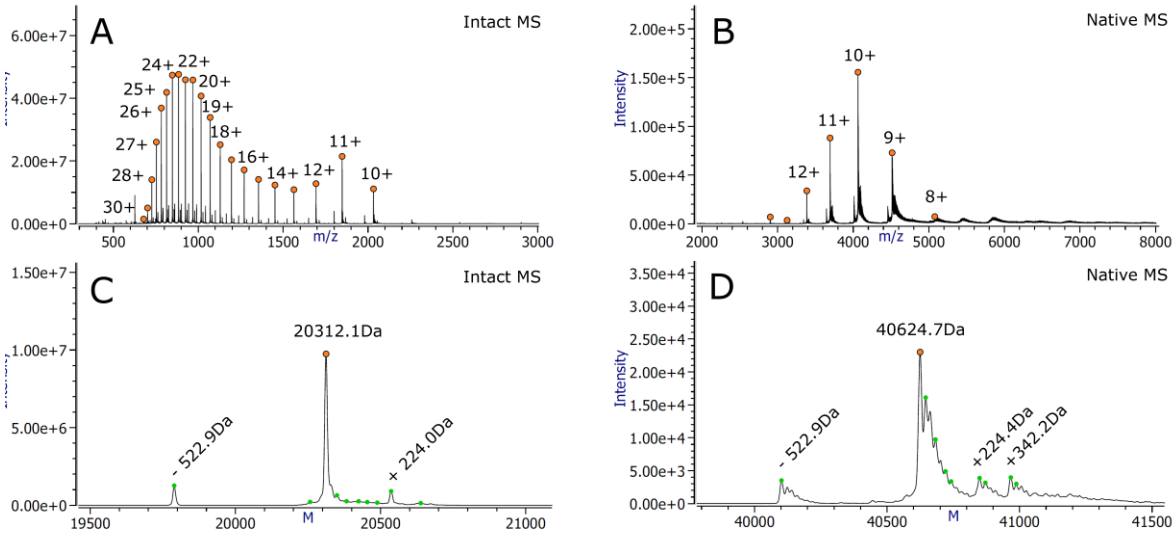
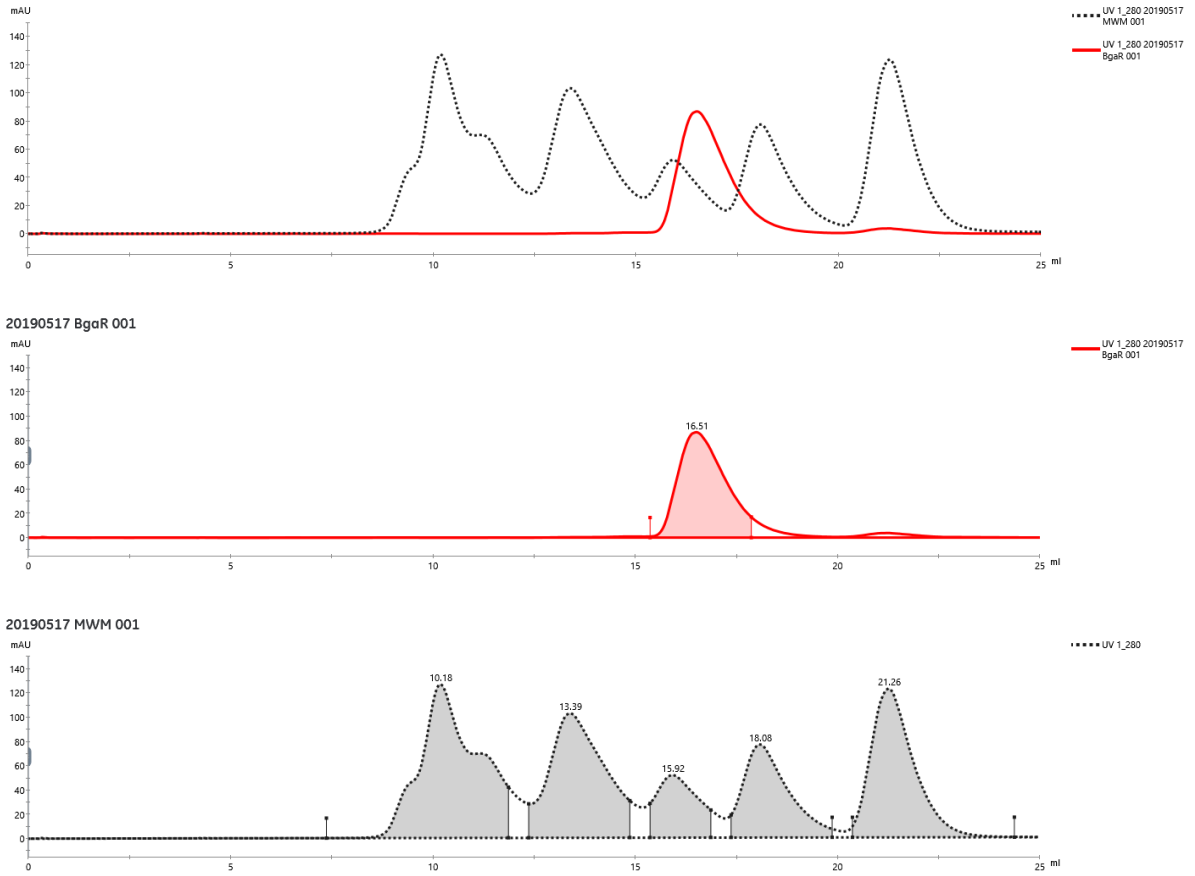


Figure S2 Mass spectrometric analysis of purified, recombinant Bga. Mass spectra of purified, recombinant BgaR under denaturing conditions (A, C – Intact MS) or native BgaR dimer under non-denaturing conditions (B, D – Native MS). Consistent with loss of the C-terminal 6xHis-tag, the accurate mass of the major intact BgaR isoform (20312.1Da) matches the calculated molecular weight of BgaR residues Met1-Glu175 (C). Minor masses are consistent with additional C-terminal truncations at residues His170 (-522.9Da) or His177 (+224.0Da). The deconvoluted native mass spectrum of BgaR confirms that the purified, recombinant protein forms a stable homodimer in solution (D). Minor masses are consistent with C-terminal truncations (-522.9Da, +224Da) or binding of lactose to the BgaR homodimer (+342.2Da).



Molecular weight markers

Component	MolecularWeight*	Retention (mL)
Thyroglobulin (bovine)	670,000	10.18
γ-globulin (bovine)	158,000	13.39
Ovalbumin (chicken)	44,000	15.92
Myoglobin (horse)	17,000	18.08
VitaminB12	1,350	21.26

Figure S3 Size exclusion chromatography (Superdex 200). $BgaR_{1\text{-}170}$ runs close (16.5 mL compared to 15.9 mL) to Chicken Ovalbumin (44 kD); assuming a linear plot gives a calculated molecular weight of 36-37 kD. These data indicate that BgaR is likely a dimer (about 40.5 kD from mass spec) in solution under these conditions.