# Supporting Information for:

# A periplasmic binding protein dimer has a second allosteric event tied to ligand binding

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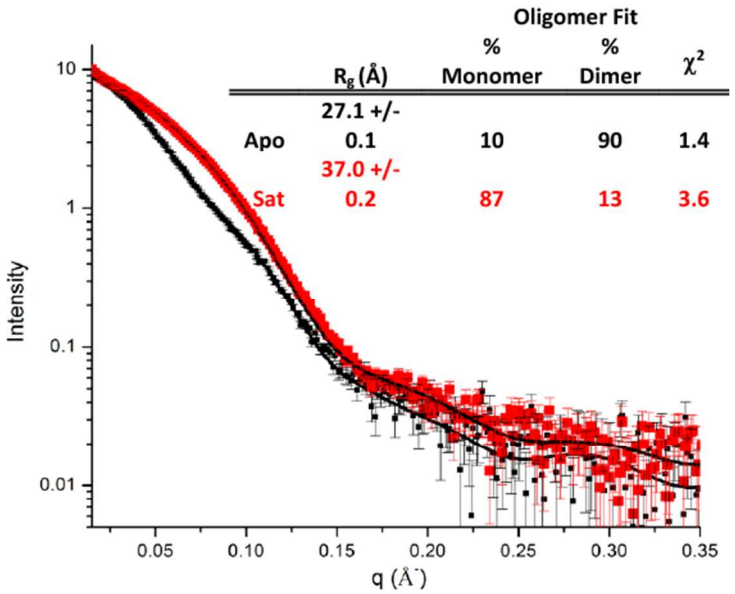


**Figure S1.** SAXS of tmMnBP3 at 65˚C. SAXS curves of apo (black) and ligand bound tmMnBP3 (red) collected at 65˚C. OLIGOMER fitting of experimental data as a mixture of monomer and dimer are solid lines. The Rg and the OLIGOMER calculated percentage of monomer and dimer in each sample are shown.

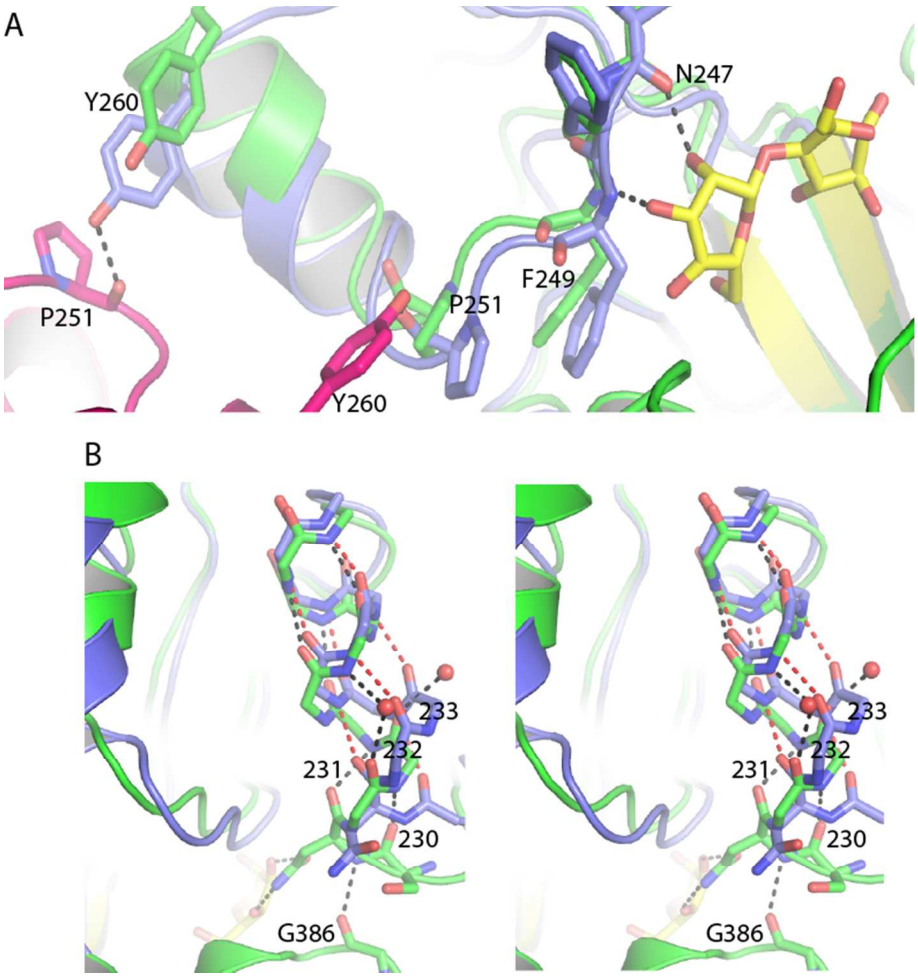


**Figure S2.** The tmMnBP3 allosteric switch. (A) Close-up view of the ligand induced changes in the B/B' helix. Ligand binding leads to a shifting of the B/B' helix and dissociation of the hydrogen bonds between Tyr260 and Pro251. Mannobiose bounds structure is shown in green ribbon model with mannobiose colored yellow. The two monomers of the homodimeric tmMnBP6 are colored in magenta and blue with the mannobiose bound structure superimposed on only the magenta monomer for clarity. (B) Stereo view of the ligand induced changes in the A/A' helix, colored as in panel A. Hydrogen bonds in the ligand bound form are shown as black dashed lines whereas hydrogen bonds are represented as red dashed lines in the apo protein.

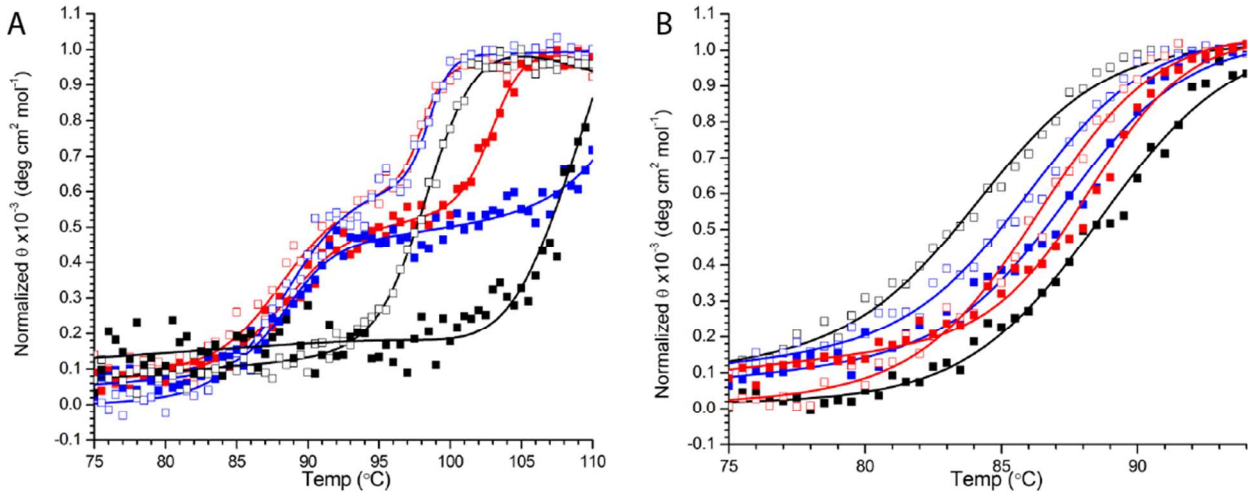


**Figure S3.** Ligand binding to wild-type and mutant tmMnBP proteins. (A) Thermal melting of tmMnBP3 in 2 M guanidine hydrochloride monitored by circular dichroism (CD). Black line, wild-type tmMnBP3; Blue line, M239R tmMnBP3; Red line N231A tmMnBP3. Wild-type is a fit to a two-state model and mutants are a fit to a three-state model. Open squares are apo protein, solid squares are in the presence of 0.5 mM mannobiose. (B) Thermal melting of tmMnBP6 in 2 M guanidine hydrochloride monitored by CD. Black line, wild-type tmMnBP6; Blue line, M239R tmMnBP6; Red line N231A tmMnBP6. Solid lines are a fit to a two-state model. Open squares are apo protein, solid squares are in the presence of 0.5 mM mannobiose.

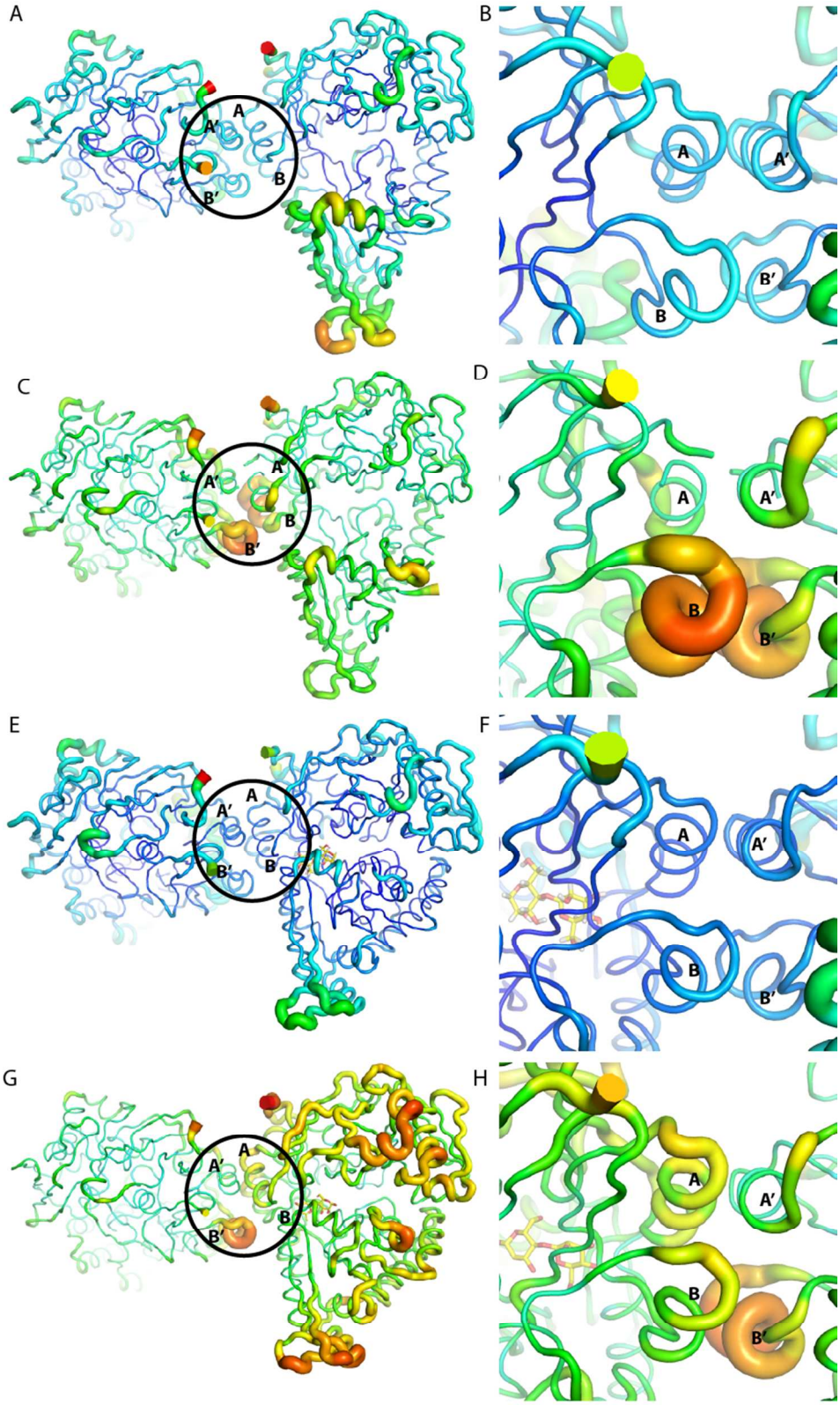
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**Figure S4.** Dynamics of the tmMnBP6 protein interface. (A) Overall structure of the tmMnBP6 ApoDimer, where r.ms.f. from molecular dynamics simulations is represented by the width of the backbone. Interface helices are indicated. (B) Close-up view of panel A. (C) Overall structure of the tmMnBP6 ApoDimer, where atomic B-factors are represented by the width of the backbone. (D) Close-up view of panel C. (E) Overall structure of the tmMnBP6 MixHetDimer, where r.ms.f. from molecular dynamics simulations is represented by the width of the backbone. (F) Close-up view of panel E. (G) Overall structure of the tmMnBP6 MixHetDimer, where atomic B-factors are represented by the width of the backbone. (H) Close-up view of panel G.

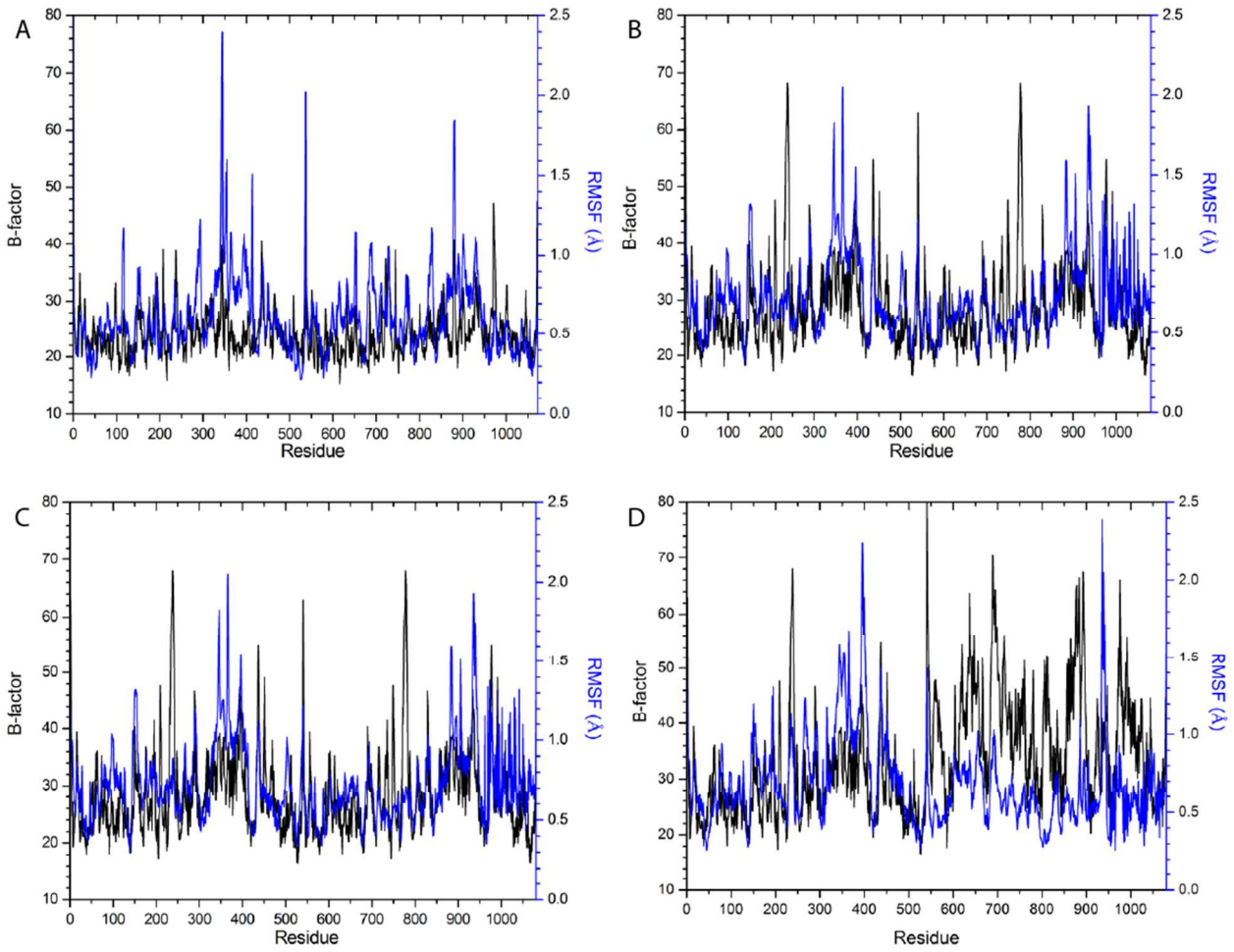


**Figure S5.** Dynamics and B-factors of the tmMnBP proteins. (A) tmMnBP3 apo homodimer. Monomers are from residue 1-536 and 537-1072. (B) tmMnBP3 MixHetDimer model. Residues 1-536 are a monomer from the apo homodimer and residues 537-1073 are the mannobiose bound monomer (C) tmMnBP6 apo homodimer. Monomers are from residue 1-540 and 541-1080. (D) tmMnBP6 MixHetDimer model. Residues 1-540 are a monomer from the apo homodimer and residues 541-1080 are the mannobiose bound monomer.

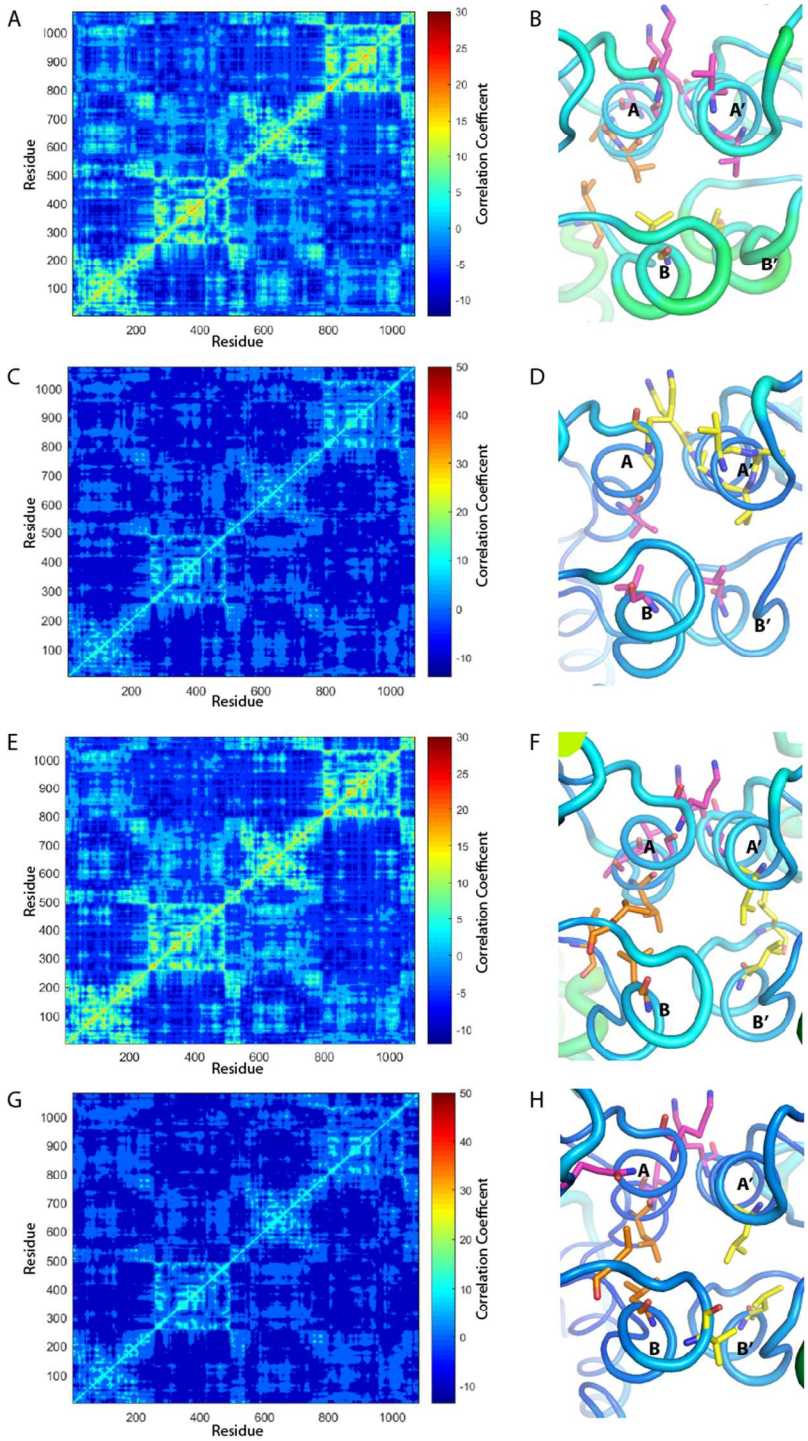


**Figure S6.** Dynamically coupled residues in tmMnBPs. (A) Apo tmMnBP3 homodimer normalized correlation matrix. (B) Close-up view of correlated residues in Apo tmMnBP3 homodimer interface. (C) tmMnBP3 MixHetDimer model normalized correlation matrix. (D) Close-up view of correlated residues in the tmMnBP3 MixHetDimer model interface. (E) Apo tmMnBP6 homodimer normalized correlation matrix. (F) Close-up view of correlated residues in Apo tmMnBP6 homodimer interface. (G) tmMnBP6 MixHetDimer model normalized correlation matrix. (H) Close of view of correlated residues in the tmMnBP6 MixHetDimer

model interface. Networks of correlated residues are shown as stick representation and colored similarly. See methods section for details how these network residues were identified. Note the correlation matrix was normalized by subtracting the mean and dividing by standard deviation, for highlighting the significant correlated pairs.

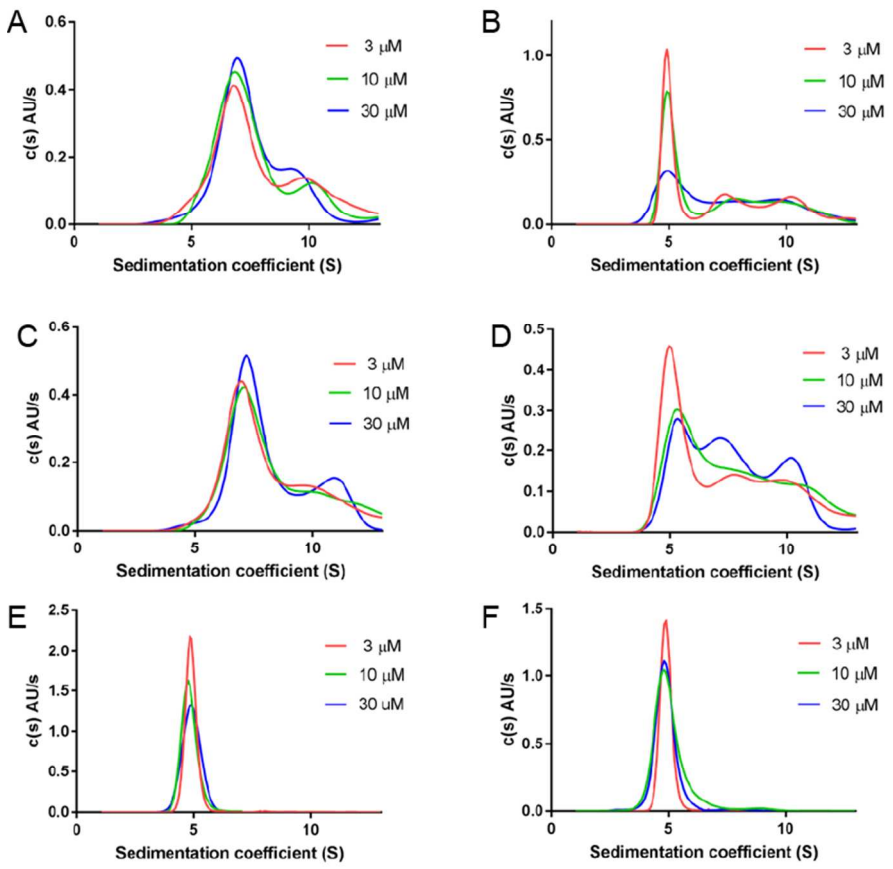


**Figure S7.** Analytical ultracentrifugation of tmMnBP6. Distribution plots of sedimentation velocity data at three protein concentrations of (A) wild type tmMnBP6, (B) wild type tmMnBP6 with mannobiose, (C) tmMnBP6 N231A, (D) tmMnBP6 N231A with mannobiose, (E) tmMnBP6 M239R and (F) tmMnBP6 M239R with mannobiose. The data are normalized by loading signal for comparative purposes.