Structure, Volume *26*

Supplemental Information

A Hotspot for Disease-Associated Variants of Human PGM1 Is Associated with Impaired Ligand Binding and Loop Dynamics

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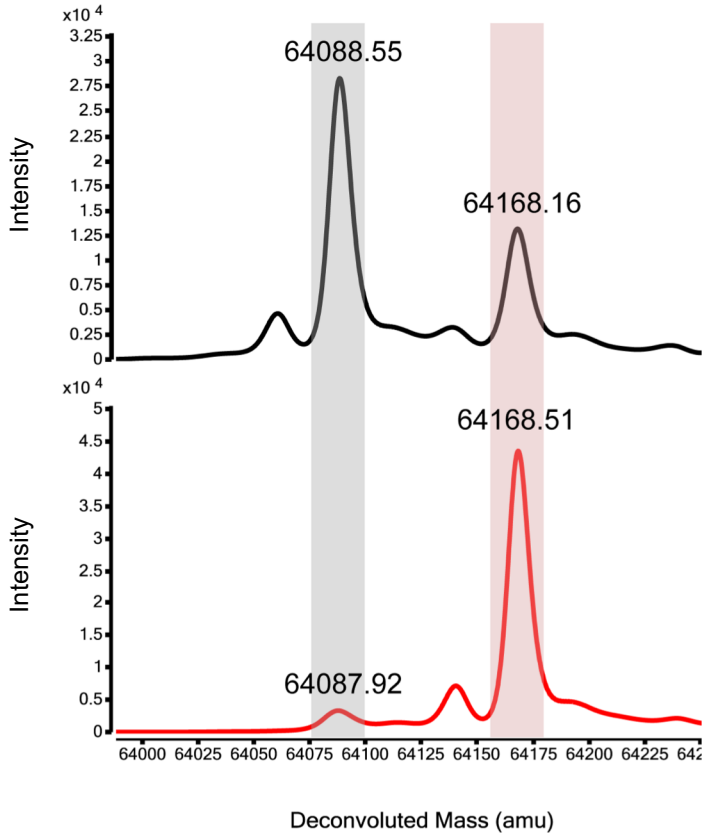


Fig. S1. Representative electrospray ionization mass spectra for the R503Q missense variant, related to Methods. The two major peaks are for phospho-enzyme (red shading) and dephospho-enzyme (gray shading). Spectra are shown for the protein as purified (top panel) and after treatment with glucose 1,6-bisphosphate (bottom). Spectra of other variants were highly similar.

Fig. S2

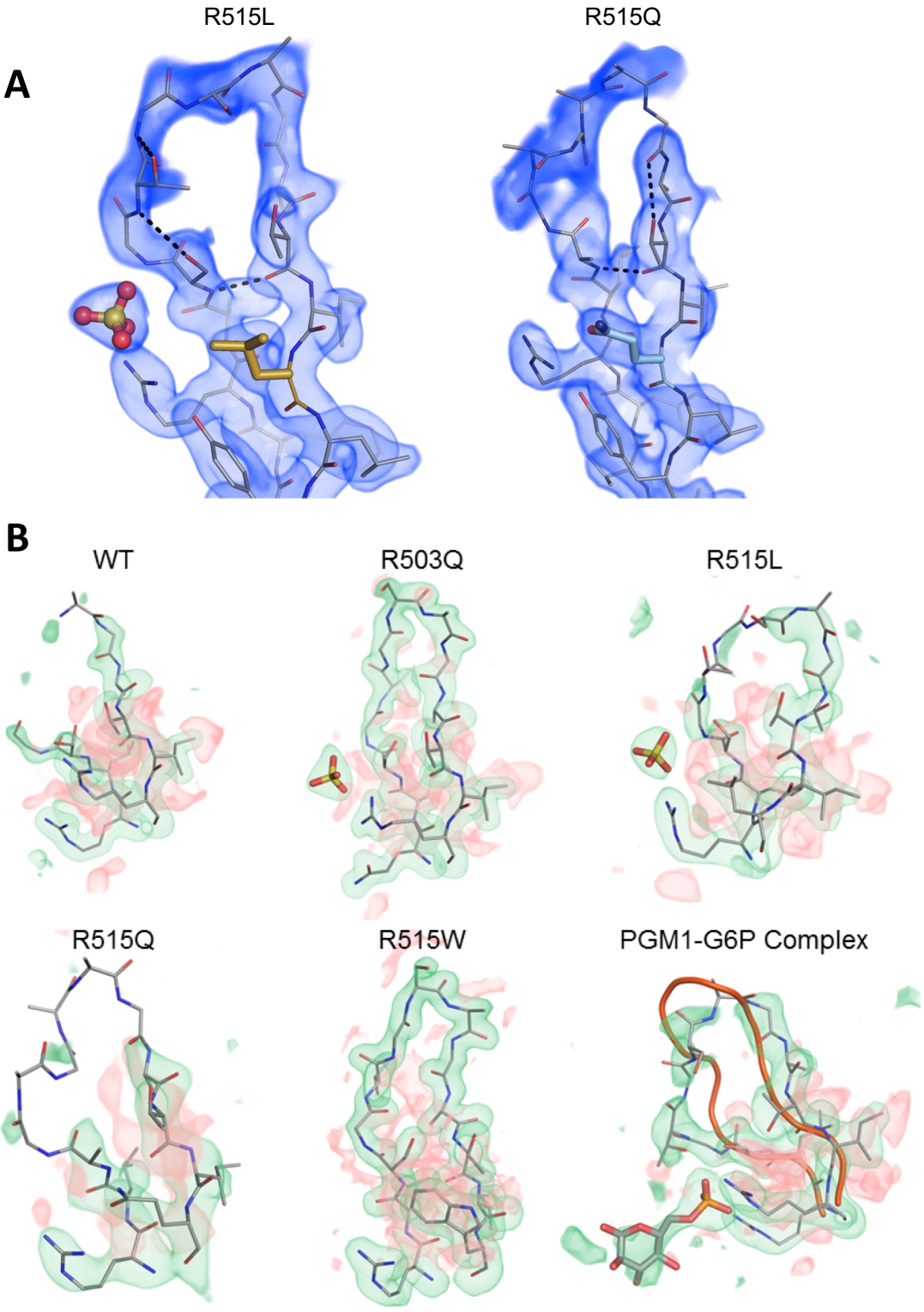
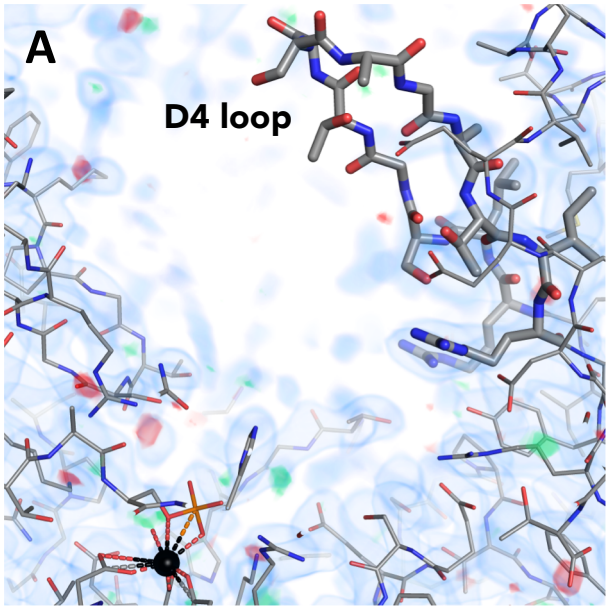
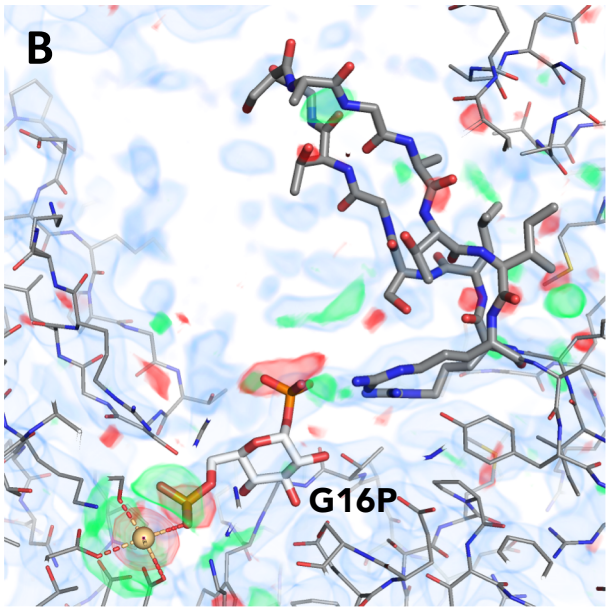


Fig. S2. Electron density maps in the vicinity of the D4 loop of PGM1, related to Fig. 2 and 3. (*A*) Final $2F_o$-F_c electron density maps contoured at 1.0 σ for the D4 loop of missense variants R515L (*left*) and R515Q (*right)*; backbone interactions are indicated by dashed lines. Compare with Fig. 2C-E in text. (*B*) Omit maps of the D4 loop for WT PGM1 (5EPC), the four missense variants, and the PGM1-G6P complex. Positive (green) and negative (red) electron density at 3.0 σ is shown for residues 503-515 and the ligand, when present. For the G6P complex, the D4 loop (orange) from the R503Q variant is superimposed, highlighting differences with the ligand-bound conformer. Omit map density at this contour level is clear for the two high resolution structures, R503Q and R515W, and moderate for the R515L structure. Weak density for the R515Q loop may be due to the lower resolution and higher overall B-factor of this data set (Table 2). Both positive and negative density is shown for completeness; no negative density is found on atoms of the structures.





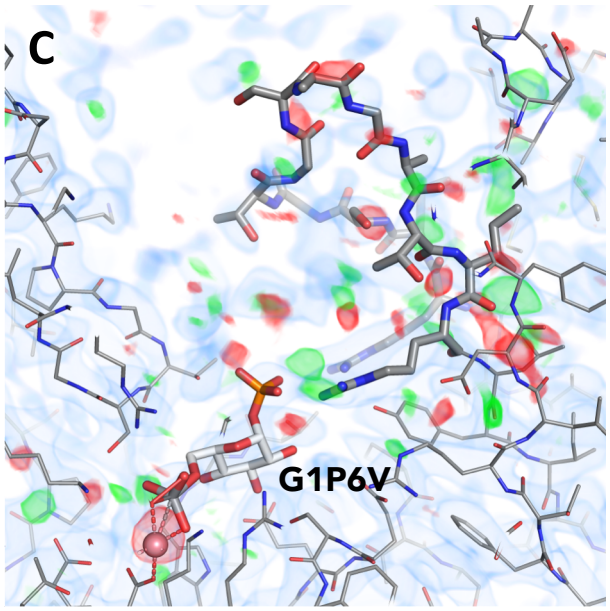


Fig. S3. Electron density maps of the D4 loop and active site vicinity of rabbit PGM, related to Fig. 3. (*A*) Apo-enzyme (PDB ID 3PMG, 2.4 Å resolution). (*B*) Enzyme complex with glucose 1,6-bisphosphate (PDB ID 1C47, resolution 2.7 Å). (*C*) Enzyme complex with glucose 1-phosphate-6-vanadate, resolution 2.7 Å). 2Fo-Fc (light blue) and Fo-Fc (green and red) maps are shown at contours of 1.0 and ±3.0 σ , respectively. Ligands and D4 loop are shown in sticks. All three structures lack supporting density for the model of the D4 loop; multiple other problems in the maps are apparent, including poor density for the entire C-terminal domain of both complexes (not shown). Further examination can be done using the one-click electron density visualization tools on the structure summary page of each deposition at the RSCB PDB.

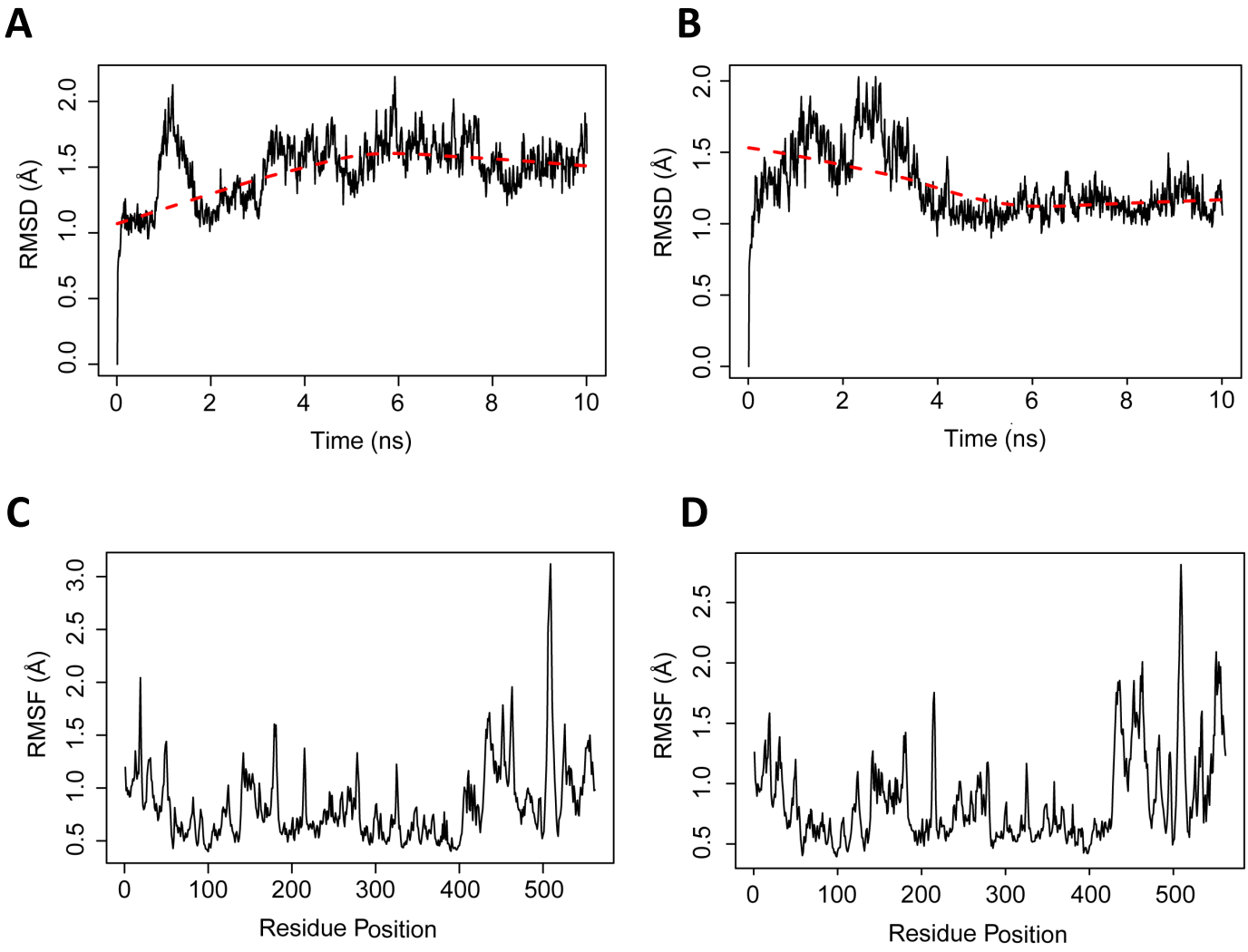


Fig. S4. Analysis from 10 ns MD simulations of WT PGM1 and the R503Q missense variant, related to Fig. 4. The root mean square deviation (RMSD) of the Cα atoms is shown with respect to the initial MD frame for (*A*) WT PGM1 and (*B*) the R503Q variant. The moving average is shown as a dashed red line. The per-residue root mean square fluctuations (RMSF) for (*C*) WT PGM1 and (*D*) the R503Q missense variant calculated after superposing each frames' coordinates to the first frame.