Supplemental Materials and Methods

Production, crystallization and data collection of selenomethione-substituted BaPNGM. Expression, purification, and crystallization of native BaPNGM has been previously described (*1*). Production of selenomethionine (SeMet) substituted protein was carried out as for the native protein, with the following modifications of the method described in (*2*). Briefly, a small scale culture of *E. coli* BL21(DE3) cells containing the expression plasmid for BaPNGM was grown in LB media with 100 µg/mL ampicillin, centrifuged, and the cell pellet re-suspended in M9 minimal media (containing M9 salts, 2 mM $MgSO_4$, 0.4% w/v, glucose and 0.1 mM $CaCl_2$) with ampicillin (100 µg/mL final concentration) and thiamine (0.00005% w/v final concentration). A one-liter culture was grown at 37º C to an absorbance of 0.8 at 600 nm. 10 mL of a solution with various amino acids and selenomethionine (Sigma) (containing 50 mg selenomethionine, 100 mg lysine hydrochloride, 100 mg threonine, 100 mg phenylalanine, 50 mg leucine, 50 mg isoleucine and 50 mg valine in 10 mL of 50 mM phosphate buffer, pH 7.5) was added to the cultures, which were grown for another 15 minutes. The flask was chilled for 30 minutes at 4º C and IPTG added to a final concentration of 0.4 mM. The culture was grown for an additional 24 hours at 21ºC before harvesting by centrifugation. Lysis and purification were as previously described (Mehra-Chaudhary et al, 2009). Incorporation of selenomethionine was verified by MALDI-TOF mass spectrometry (data not shown).

The SeMet protein crystallized isomorphously under the same conditions with the native protein and the same cryoprotectant was employed (*1*). A medium resolution data set (3.2 Å resolution) was collected for SeMet PNGM from a single crystal on beamline

4.2.2 at the Advanced Light Source of Berkeley National Laboratory using a NOIR-1 CCD detector. Diffraction data were processed using d*TREK (*3*) and are summarized in Table S1.

Multiple sequence alignments. Sequences for PNGM enzymes used in alignments were obtained from the Protein Information Resource or PIR (http://pir.georgetown.edu/) (*4*) and compiled from proteins with PIRSF ID 005849. Sequences that were not ~450 residues in length (i.e., fragments) were removed from the compilation. Sequences with >97% identity to others were removed automatically in Jalview, and the resulting compilation contains 171 sequences. Multiple sequence alignments were performed with MUSCLE (*5*), and displayed with Jalview (*6*).

Table S1. Data collection statistics for SeMet substituted BaPNGM crystals.

Wavelength (Å)	0.978841
Space group	$P3_221$
Unit cell dimensions (Å)	a = b = 85.7, c = 270.1
Mosaicity (°)	0.97
Resolution range (Å)	32.53 – 3.20 (3.31-3.20)
Total no. reflections	19,849
Avg. redundancy	9.7 (10.3)
Completeness	100.0 (100.0)
R_{merge} (%)	13.5 (47.6)
I/σ(I)	8.3 (3.3)

References

1. Mehra-Chaudhary, R., Neace, C. E., and Beamer, L. J. (2009) Crystallization and initial crystallographic analysis of phosphoglucosamine mutase from Bacillus anthracis, *Acta Crystallogr Sect F Struct Biol Cryst Commun 65*, 733-735.
2. Van Duyne, G. D., Standaert, R. F., Karplus, P. A., Schreiber, S. L., and Clardy, J. (1993) Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin, *Journal of molecular biology 229*, 105-124.
3. Pflugrath, J. W. (1999) The finer things in X-ray diffraction data collection, *Acta Crystallogr D Biol Crystallogr 55 (Pt 10)*, 1718-1725.
4. Wu, C. H., Yeh, L. S., Huang, H., Arminski, L., Castro-Alvear, J., Chen, Y., Hu, Z., Kourtesis, P., Ledley, R. S., Suzek, B. E., Vinayaka, C. R., Zhang, J., and Barker, W. C. (2003) The Protein Information Resource, *Nucleic acids research 31*, 345-347.
5. Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic acids research 32*, 1792-1797.
6. Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., and Barton, G. J. (2009) Jalview Version 2--a multiple sequence alignment editor and analysis workbench, *Bioinformatics (Oxford, England) 25*, 1189-1191.

Supplemental figures

Fig. S1. Ribbon diagrams showing superposition of BaPNGM with related enzymes. A) The BaPNGM monomer (blue) superimposed on *P. aeruginosa* PMM/PGM (PDB ID 1P5D) shown in green. B) A superposition of the BaPNGM dimer (blue) and the dimer of *F. tularensis* BaPNGM (PDB ID 3I3W) shown in magenta.

Fig. S2. A multiple sequence alignment of eight PNGM sequences from human pathogens, with previous functional or biochemical characterization. The sequence of BaPNGM is shown at the top. The four key regions of the active site are highlighted with orange tabs, and key functional residues by red triangles. Yellow squares indicate conserved residues unique to the PNGM enzymes that may provide specificity for and/or permit binding of the glucosamine phosphosugar substrate and product. The dimerization helix is shown in green, other residues in the dimer interface are highlighted by green triangles. Red asterisks and boxes indicate conserved positions in the dimerization helix that differ from related monomeric proteins. Also included at the bottom of the alignment (below blue dashed line) is the sequence of *P. aeruginosa* PMM/PGM, a related enzyme with differing substrate specificity. Sequence identities between BaPNGM and other PNGM proteins in this alignment range from a high of 67% with *S. aureus* and a low of 40% with *F. tularensis.* BaPNGM and *P. aeruginosa* PMM/PGM have a sequence identity of 27%.

Fig. S3. A multiple sequence alignment of 171 PNGM sequences derived from PIRS005849 as described in Supporting Experimental Procedures. Extent of sequence conservation is indicated by blue shading.

Fig. S1

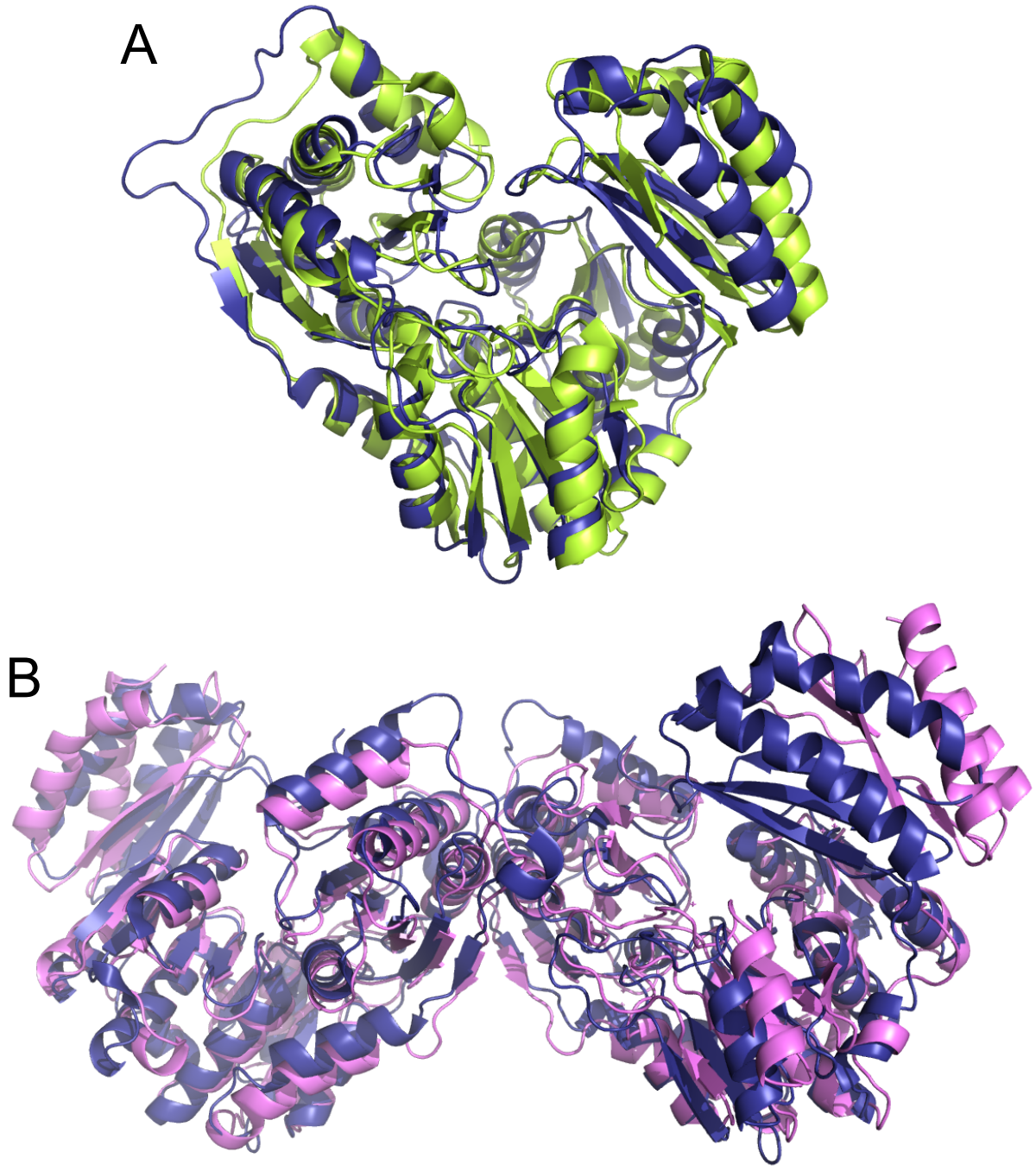
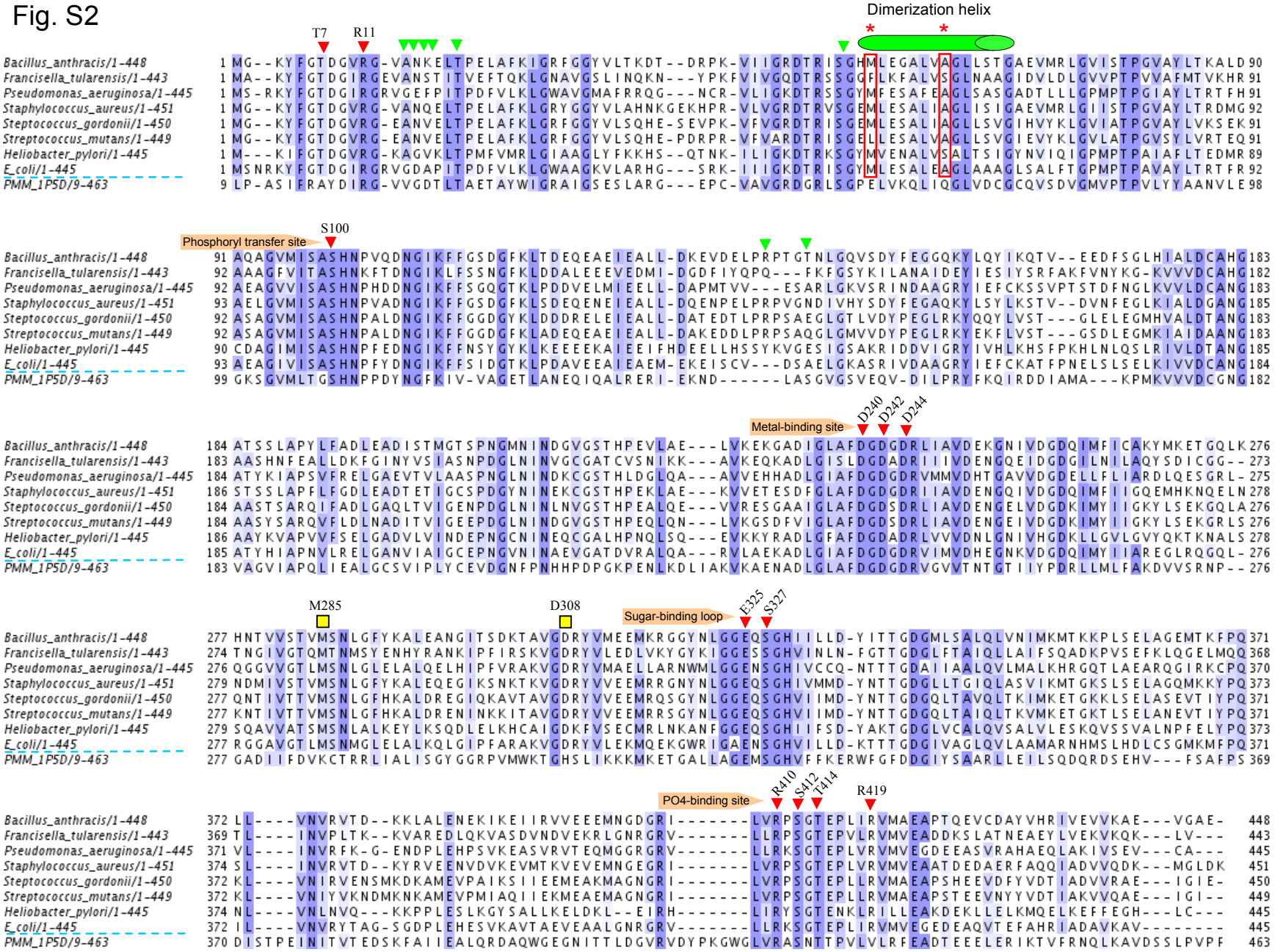


Fig. S2


Dimerization helix
T7
R11
S100
Phosphoryl transfer site
D240
D242
D244
Metal-binding site
M285
D308
Sugar-binding loop
E325
S327
R410
S412
T414
R419
PO4-binding site
Bacillus_anthracis/1-448
Francisella_tularensis/1-443
Pseudomonas_aeruginosa/1-445
Staphylococcus_aureus/1-451
Steptococcus_gordonii/1-450
Streptococcus_mutans/1-449
Heliobacter_pylori/1-445
E_coli/1-445
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