## **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  $\mu$ g/mL ampicillin, centrifuged, and the cell pellet re-suspended in M9 minimal media (containing M9 salts, 2 mM MgSO<sub>4</sub>, 0.4% w/v, glucose and 0.1 mM CaCl<sub>2</sub>) with ampicillin (100 µg/mL final concentration) and thiamine (0.00005% w/v final concentration). A one-liter culture was grown at 37° 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 4° C and IPTG added to a final concentration of 0.4 mM. The culture was grown for an additional 24 hours at 21°C 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 (I). 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*).

Wavelength (Å)	0.978841
Space group	P3 <sub>2</sub> 21
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 <sub>merge</sub> (%)	13.5 (47.6)
$I/\sigma(I)$	8.3 (3.3)

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

## References

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- 5. Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic acids research 32*, 1792-1797.
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## **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

**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.



Fia. S2		Dimerization helix
	T7 R11	
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 PMM_1PSD/9-463	1 MG KY F GT D GVR G - VANK E LT P E LAF 1 MA KY F GT D G I R G EVANST I T V E F T Q 1 MS - R KY F GT D G I R G R V G E F P I T P D F V L 1 MG KY F GT D GVR G - VANQ E LT P E LAF 1 MG - KY F GT D GVR G - E ANVE LT P E LAF 1 MG K I F GT D GVR G - KAGVK LT PMF VM 1 MS N R KY F GT D G I R G R V G D A P I T P D F V L 9 L P - AS I F R AY D I R G - VVG DT LT AET AY	K I GR F G G Y V LT K DT D R P K - V I I G R DT R I S G HM L E G A L VA G L L ST G A E VMR L G V I ST P G VAY LT KALD 90 K L G NAV G S L I NQ K N Y P K F V I V G Q DT R S S G G F L K F A L V S G L NAA G I D V L D L G V V P P V V A F M T V K H R 91 K L G WA V G MA F R R Q G N C R - V L I G K DT R S S G Y M F E S A F E A G L S A S G A D T L L L G P M P T P G I AY L T R T F H 91 K L G W Y G Y V L A H N K G E K H P R - V L V G R D T R V S G E M L E S A L I AG L I S I G A E V M R L G I I S T P G V AY L T R T F H 91 K L G R Y G G Y V L A H N K G E K H P R - V L V G R D T R V S G E M L E S A L I AG L I S I G A E V M R L G I I S T F G V AY L T R D M G 92 K L G R F G G Y V L S Q H E - S E V P K - V F V G R D T R I S G E M L E S A L I AG L L S V G I H V Y K L G V I A T P G V AY L V K S E K 91 K L G R F G G Y V L S Q H E - P D R P R - V F V G R D T R I S G E M L E S A L I AG L L S V G I H V Y K L G V L A T P G V S Y L V K T E Q 91 R L G I A A G L Y F K K H S Q T N K - I L I G K D T R K S G Y M V E N A L V S A L T S I G Y N V I Q I G P M P T P A I A F L T E D M R 89 K L G W A A G K V L A R H G S R K - I I I G K D T R I S G Y M V E N A L V S A L T S I G Y N V I Q I G P M P T P A V AY L T R T F R 92 W I G R A I G S E S L A R G E P C - V A V G R D G R L S G P E L V K Q L I Q G L V D C G C Q V S D V G M V P T P V L Y A A N V L E 98
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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 PMM_1PSD/9-463	92 AAAG FV IT AS HNK FT DNG I KLFSSNGF 92 AEAG VV I SASHNPHDDNG I KFFSGQGT 93 AELG VM I SASHNPVADNG I KFFGGDGF 92 AS AG VM I SASHNPALDNG I KFFGGDGY 92 AS AG VM I SASHNPALDNG I KFFGGDGF 90 CDAG I M I SASHNPFEDNG I KFFSVGY 93 AEAG I V I SASHNPFYDNG I KFFSIDGT 99 GKSGVMLTGSHNPPDYNGFK I V-VAGE	K LDDAL E E E VEDMI – DGDF I Y QPQ – – – FK F GS Y K I LANA I DEY I ES I Y SR FAK F VNY KG – K VV VD CAH G 182 K LPDD VELMI E E LL – DAPMT VV – – ES AR L GK VS R I NDAAGRY I E F C KS S VPT ST D F NG L K V V LD CAN G 183 K LS D E Q E N E I E A LL – D Q E N P E L P R P V G N I V H Y S D Y F E G A Q KY L S Y L KS T V – – D V N F E G L KI A L D G AN G 185 K LD D D R E L E I E A LL – D A T E D T L P R P S A E G L G T V D Y P E G L R KY Q Q Y L V S T – – G L E L E G M H V A L D T AN G 183 K LAD E Q E A E I E A L L – D A K E D D L P R P S A Q G L G M V V D Y P E G L R KY Q Q Y L V S T – – G L E L E G M H V A L D T A N G 183 K LAD E Q E A E I E A L L – D A K E D D L P R P S A Q G L G M V V D Y P E G L R KY E K F L V S T – – G S D L E G M K I A I D A A N G 183 K L K E E E KA I E E I F H D E E L L H S Y K V G E S I G S A K R I D D V I G R Y I V H L K H S F P K H L N L Q S L R I V D T A N G 184 K L P D A V E E A E E A E M – E K E I S C V – – – D S A E L G KA S R I V D A A G R Y I E F C K A T F P N E L S L S E L K I V V D C A N G 184 T LAN E Q I Q A L R E R I – E K N D – – – – L A S G V G S V E Q V – D I L P R Y F K Q I R D I A MA – – – K P M K V V D C G N G 182
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