**Fig. S-1. CD14 enhances recognition of a TLR2 agonist carried by NA-LprG.** (A) HEK293.TLR2/CD14 and HEK293.TLR2 cells show a dose-dependent IL-8 response to LprA, LprG, and NA-LprG, but no response to NA-LprA. Control HEK293 cells lacking TLR2 and CD14 failed to respond to all four proteins (data not shown). IL-8 production was quantified by ELISA, and data are reported as the mean +/- SD of triplicate HEK293.TLR2 assays. Results are representative of at least 3 independent experiments.
**Fig. S-2.** NA-LprG activity is dependent on TLR1 and TLR2, similar to triacylated lipopeptides and glycolipids. Bone marrow-derived macrophages from TLR2−/−, TLR1−/−, TLR6−/− or wild-type mice were incubated for 12-16 h with NA-LprG, glycolipid or lipopeptide. TNF-alpha production was determined by ELISA. (A) NA-LprG was purified by Ni-affinity and anion-exchange chromatography from *M. smegmatis*. (B-D) NA-LprG was purified from *E. coli* by Ni-affinity and anion-exchange chromatography, incubated with Mtb H37Ra lysate (B), LAM from *M. smegmatis* (C) or LM from *M. smegmatis* (D), and then repurified by Ni-affinity and ion exchange chromatography. (E) Response to diacylated FSL-1 lipopeptide (model TLR2/TLR6 agonist). (F) Response to triacylated Pam₃CSK₄ lipopeptide (model TLR2/TLR1 agonist). Response to a TLR9 agonist (CpG ODN 1826 TLR9 agonist) was intact in all knockout cells (data not shown). Data are reported as mean +/- SD of triplicate macrophage assays.
Fig. S-3. Collision induced dissociation mass spectrometry (CID-MS) of ligands eluting from NA-LprG. (A-C) Component structures of parental ions at m/z 851.5 (PI), 1013.5 (PIM₁), and 1413.7 (Ac₁PIM₂), corresponding to the structures in Fig. 5. (D) Purified NA-LprG (24 μg) and a control protein, PabC (26 μg), were denatured with 1 ml methanol for 5 min, followed by vortexing; 10 μL of methanol eluate was loaded onto a nanospray tip for negative-mode electrospray ionization mass spectrometry (Thermo Finnigan LCQ Advantage). NA-LprG yielded ions corresponding to phosphatidylinositol (PI at m/z 851.5) and triacyl PIM₂ (Ac₁PIM₂ at m/z 1413.7), similar to results seen in Fig. 5. These species were not associated with PabC.
Fig. S-4. LprG, LprA and LppX are homologous lipoproteins with hydrophobic pockets that represent putative ligand binding sites. A structural homolog search (VAST) of LprG against the PDB database revealed only one protein, Mtb LppX, with a structural fold very similar to LprG.
LppX is also a lipoprotein of Mtb, and *lppX* is part of a gene cluster thought to be involved in PDIM biosynthesis. The structure of LppX shows a larger cavity that may contain three fatty acids (two C18 and one C22), although the physiologically relevant ligands remain unclear. A detailed structural comparison of LppX and LprG is provided in Supplemental Fig. S-4. In addition, we model the structure of LprA, which has homology to LprG.

Conserved residues are shown (in orange on the LprG structure) for comparison of LprG with LppX (A) and LprA (B). Based on sequence alignment in ClusterW, LprG has 28% identity with LppX and 34% identity with LprA. Relative to LprG, LprA shows greater conservation of residues that contribute to the cavity structure. Differences in the residues near helices α2, α3 and α4, which cover the front of the binding pocket and keep the entrance to the side in LprG, results in access to the LppX pocket at the front. (C) Superimposition of LprG (dark blue) and LprA (pink, modeled from LprG). (D) Superimposition of LprG (dark blue) and LppX (light blue). Side chains are shown for residues on helices α2, α3 and α4 that are conserved between LprG and LprA. Since six out of seven residues on helix α4 are conserved, the entrance of LprA is predicted to be similar to that of LprG (on the side, not at the front). (E, F) Hydrophobic surface slab view of LprA model (E) and LppX (F) (white, carbon; red, oxygen; blue, nitrogen; yellow, sulfur). LprA and LppX have a folded structure and large hydrophobic cavity at the center of the molecule, as seen with LprG.

LprG is a member of a family of lipoproteins found in mycobacteria that have been predicted to function in cell wall biosynthesis. Included in the family are LprA and LppX. LppX shares 28% sequence identity with LprG, and they have similar α/β fold structures with large hydrophobic cavities (Sulzenbacher et al., 2006) (Fig. S-4B, F). Access to binding pocket, however, is significantly different for LprG and LppX (Fig. S-4D). In LprG, helices that cap the pocket from one side are at the center of the β-sheet. In LppX, these residues form a flexible loop and push the adjacent helix α3 to block entrance to the cavity at the site provided in LprG, shifting the flexible loop in LppX outward from the cavity, creating a large binding pocket. The binding pocket in the LppX crystal structure was found to contain two C18 fatty acids and one C22 fatty acid, although the physiological relevant ligand from *M. tuberculosis* is not known. The residues that are conserved between LppX and LprG are dispersed, although the residues in helices 2, 3 and 4 are more conserved between LprG and LprA relative to LppX (Fig. S-4C), implying that LprA may be closer to LprG than LppX in terms of structure of the cavity and its entrance. However, the different residues in the cavity of LprA could produce differences in cavity shape and size not revealed by this modeling.
Fig. S-5. LAM is associated with acylated LprG in *M. smegmatis*. Since biochemical analysis of LAM association in prior figures was done exclusively with NA-LprG, we assessed whether LAM is also associated with acylated LprG when expressed in *M. smegmatis*. NA-LprG was purified from *M. smegmatis* and *E. coli*, and acylated LprG was purified from *M. smegmatis*. Protein preparations (3 µg) were analyzed by SDS-PAGE and Western analysis as in Fig. 5. Anti-6xHis Western analysis and Ponceau stain confirmed presence of each protein at the predicted apparent molecular weight. Anti-LAM Western analysis showed that LAM was not associated with NA-LprG from *E. coli*, a predicted negative control result. LAM was detected in association with acylated LprG as well as NA-LprG from *M. smegmatis*. Moreover, LAM association with acylated LprG was higher than with NA-LprG, likely due to more efficient targeting of acylated LprG to periplasmic/cell wall location where LAM is localized. Prior to this experiment, one consideration was that acyl chains of LprG might compete for binding to the LprG pocket, potentially producing lower LAM association with acylated LprG than with NA-LprG. These results confirm that acylated LprG binds LAM when expressed in *M. smegmatis*. 
Supplementary Methods

Mammalian cell culture

Unless otherwise specified, incubations with eukaryotic cells were performed at 37°C in a 5% CO₂ atmosphere. Standard medium was DMEM (HyClone, Logan, UT, ASK30773) supplemented with 10% heat-inactivated FCS, 50 μM 2-ME, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, pH 7.4, and penicillin/streptomycin (HyClone). Stimulation medium was standard medium with serum concentration reduced to 0.2% FBS. Female C57BL/6J mice (8-16 weeks old) were obtained from the Jackson Laboratory, housed under specific pathogen-free conditions and used to produce macrophages. Bone marrow was obtained from TLR1⁻/⁻, TLR2⁻/⁻ and TLR6⁻/⁻ mice courtesy of Amy Hise, Case Western Reserve University; these mice were generously provided by Shizuo Akira (Research Institute for Microbial Disease, Osaka University, Osaka, Japan) and were back-crossed to C57BL/6J mice a minimum of eight times. CD14 knockout mice (B6.129S-Cd14⁻/⁻) were obtained from the Jackson Laboratory and were compared to both C57BL/6J mice and F2 hybrids of C57BL/6J and 129sv. Bone marrow cells were cultured for 7-12 d in standard medium supplemented with 25% LADMAC cell-conditioned medium. HEK293 cells stably expressing TLR2-YFP (HEK293.TLR2) were produced previously. HEK293 cells (ATCC CRL-1573) were stably transfected with the empty vector to produce a control HEK293.pcDNA3 cell line. Transfected HEK293 cell lines were maintained in HEK medium (DMEM supplemented with 10% heat-inactivated FCS (HyClone)) supplemented with ciprofloxacin (10 μg/ml) and...
geneticin (500 μg/ml). HEK293.TLR2-CD14 cells were purchased from Invivogen (293-htlr2cd14) and cultured in HEK medium supplemented with 100 μg/ml Normocin (Invivogen, 100 μg/ml Hygromycin B and 10 μg/ml Blasticidin).

**Cytokine ELISAs**

HEK293 cells were incubated in 96-well plates (20,000 cells/well) in 90 μl of stimulation medium for 5-8 h and then for an additional 16 h with or without TLR2 agonist. Supernatant IL-8 concentration was quantified by ELISA (R&D, Minneapolis, MN, DY208). Bone marrow-derived macrophages were incubated overnight at 100,000 cells/well in standard medium and then for 12 h in stimulation medium with or without agonist. Supernatants were collected and stored at -80°C. TNF-alpha in the supernatant was quantified by ELISA (BD Biosciences #558874, R&D DY410). The following synthetic TLR agonists were also used: FSL-1 (Invivogen, tlrl-fsl), Pam3CSK4 (Invivogen, tlrl-pms), and CpG-B ODN 1826 (5'-tcc atg acg ttc ctg acg tt-3' lot C44-05225-q1a) provided by Coley Pharmaceutical Group (Wellesley, MA).

**Cloning and expression of His<sub>6</sub>-tagged proteins**

LprA and NA-LprA were cloned previously. LprG was amplified from Mtb H37Rv genomic DNA by PCR using the following oligodeoxynucleotide primers (sequences written 5’ to 3’; underlined portions are NdeI and HindIII restriction enzyme recognition sites): the 5’ primer

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GCATATCCATATGCGGACCCCCAGACGCCACTG
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and the 3’ primer

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GTACAAGCTTGTACCGCAGGGGGCTTCG
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A non-acylated (NA) variant of LprG was cloned by using a 5’ primer that excluded the signal sequence and changed
the acylated cysteine to a methionine. NA-LprG was cloned with the 5’ primer GCAATTCCATATGTCGTCGGGCTC and the 3’ primer GTACAAGCTTGCTACCGGGGGCTTCG. Fusions of NA-LprA and NA-LprG were produced by digestion of the NA-Lpr constructs with Ndel, Mscl, and HindIII, and ligating the 5’ fragment of NA-LprA with the 3’ fragment of NA-LprG to make the A:G fusion protein. For the G:A fusion, the same digest was performed, and 5’ NA-LprG was fused with the 3’ NA-LprA fragment. Site-directed mutagenesis of NA-LprG was performed using the Quikchange site directed mutagenesis kit (#200519; Stratagene, La Jolla, CA) with the 5’ primer GCCGCGACGGGAAAACCTGGAAGCTCACGCTGGGT and the 3’ primer ACCCAGCGTGAGCTTCCAGTTTCCCCGTCGCGGC. For expression in *M. smegmatis*, constructs were digested with Ndel and HindIII (NEB, Ipswich, MA) and ligated into the shuttle vector pVV16 (provided by J. Belisle, Colorado State University, Fort Collins, CO) behind the constitutively active hsp60 promoter and in-frame with a C-terminal His$_6$ tag. For expression in *E. coli* Rosetta cells (#71405, EMD, Gibbstown, NJ), constructs were digested with Ndel and HindIII and ligated with the expression plasmid pET-22b(+) (Novagen, Madison, WI) (removing the pelB leader sequence), placing the coding sequence behind the IPTG-inducible T7 promoter and in frame with a C-terminal His$_6$ tag. All constructs were verified by sequencing and analyzed using Clone Manager (SciEd Software, Cary, NC). For expression in *M. smegmatis*, strain MC$^2$ 1-2C (R. Wilkinson, Imperial College, London, U.K.) was transformed by electroporation with a Gene Pulser (Bio-Rad, Hercules, CA) set at 2.5 kV, 25 µF,
and 800 Ohms. *M. smegmatis* was cultivated in Middlebrook 7H9 broth (Difco, Lawrence, KS) supplemented with 1% casamino acids (Fisher, Pittsburgh, PA, BP1424), 0.2% glycerol, 0.2% glucose, and 0.05% Tween 80; selection was with kanamycin at 30 µg/ml. For expression in *E. coli*, chemically competent *E. coli* Rosetta was transformed and cultured in Luria-Bertani broth (LB); selection was with ampicillin (50 µg/ml) and chloramphenicol (34 µg/ml). When culture OD$_{600}$ was approximately 1.0, gene expression was induced for 2-3 h by addition of 500 nM IPTG (Invitrogen, 15529-019). Bacteria were isolated by centrifugation at 6000 g for 20 min at 4°C.

**Purification of His$_6$-tagged proteins**

Acylated and non-acylated LprG, LprA and LprG-V91W were stably expressed in both *E. coli* and *M. smegmatis*, and purified as described $^4$. Cells were resuspended in lysis buffer (2.5 ml/liter of bacterial culture) consisting of 50 mM NaH$_2$PO$_4$, 300 mM NaCl, 20 mM imidazole, pH 8.0, 2.5% protease inhibitor cocktail (Sigma P8849), 75 U/ml benzonase (Novagen, 70664-3), and 2.5 mg lysozyme (Sigma L-3790) and incubated for 15 min at 37°C. Bacteria were disrupted mechanically by 4 passages through a French press (2000 psi). Insoluble material was removed from the lysate by ultracentrifugation at 100,000 g for 1 h at 4°C, and supernatant was incubated directly with Ni beads (Qiagen, Valencia, CA, 1018244) for 2-4 h at 4°C. Ni beads were transferred to polypropylene columns, washed 3x with 25 volumes of wash buffer (50 mM NaH$_2$PO$_4$, 1 M NaCl, 20 mM imidazole, 10% glycerol, pH 8.0), and bound protein was dissociated with elution buffer (50 mM NaH$_2$PO$_4$, 300 mM NaCl, 450 mM
imidazole, pH 8.0). Samples were desalted into 20 mM Tris, pH 8.0 using PD-10 columns (GE Healthcare, Uppsala, Sweden 17-085-01), subjected to anion-exchange chromatography using quaternary ammonium columns (GE Healthcare, 17-5053-01), eluted by stepwise addition of 50, 150, 200 and 1000 mM NaCl, and concentrated using 10-kDa cutoff Centricon units (Amicon, UFC801008). Protein purity was verified by SDS-PAGE with silver stain and anti-His<sub>6</sub> Western blot; yields were estimated by BCA protein assay (Pierce, Rockford, IL, 23225). Material eluted at 50-200 mM NaCl was used for all experiments. Both acylated and non-acylated forms were readily soluble in the aqueous buffers indicated above.

Mycobacterial lysates and charging of E. coli-derived proteins

Mtb strain H37Ra (ATCC 25177) was cultured with shaking at 37°C to late log phase growth (2.5 weeks) in Mtb 7H9 broth (4.7 g/l 7H9 (Difco 271310), 5 ml/l glycerol, 0.5 ml/l Tween-80 (Sigma, St. Louis, MO, P4780) supplemented with 10% albumin/dextrose/catalase (BD, Franklin Lakes, NJ 212352). Bacilli from 100 ml late log phase culture were harvested by centrifugation at 5,000 g for 20 min at 4°C, resuspended in 5 ml culture supernatant and stored at -80°C. Frozen stocks of M. smegmatis or Mtb H37Ra were thawed and used with or without resuspension in 5 ml HBSS to prepare lysates by sonication with a Misonix Sonicator 3000 (Misonix, Farmingdale, NY) at amplitude 1 for four 15-min bursts in ice water with a temperature cut-off of 40°C. Mtb H37Rv lysates were obtained from Karen Dobos-Elder and John Belisle, Colorado State University, under NIH contract HHSN266200400091C, N01-AI-40091. Since Mtb H37Rv
lysates contained EDTA, they were passed over a Ni spin column (Qiagen #31014) to remove EDTA, and MgCl$_2$ and CaCl$_2$ were added to 1.0 mM and 1.3 mM, respectively.

For charging of *E. coli*-expressed proteins, 300-500 µg of protein (purified by Ni-affinity and anion-exchange chromatography) was incubated with mycobacterial sonicate for 4 h at 37°C with rocking. Insoluble material was pelleted, and charged proteins were repurified from the supernatant by Ni-affinity and anion-exchange chromatography. For charging with purified glycolipids, proteins similarly purified from *E. coli* (100 µg) were incubated for 3 h at 37°C with 0.5-50 µg of purified glycolipid (Invivogen, San Diego, CA; LAM-MS, LM-Ms) or lysis buffer in a total volume of 100 µl and repurified as above.

**SDS-PAGE and visualization of purified proteins and glycolipids**

Gels (13% acrylamide) were cast and run using a Tris-HCl buffer system. Proteins were visualized with Silver Stain Plus (BioRad, 161-0449EDU). Carbohydrates (including glycolipids) were visualized with Pro-Q Emerald 300 (Molecular Probes, Eugene, OR P20495) following periodate oxidation. Mycobacterial proteins and glycolipids were also visualized by conventional Western analysis with rabbit polyclonal anti-BCG (DAKO, Glostrup, Denmark; 1:30,000). For conventional Western analysis, material was transferred to PVDF membranes, which were blocked with 5% milk in PBS supplemented with 0.1% Tween-20 (PBST) for 1 h at 22°C and incubated overnight at 4°C with antibody. Blots were washed three times in PBST and incubated for 2 h at room temperature with secondary antibodies conjugated to horseradish peroxidase.
Membranes were washed three times in PBST, and reactive bands were visualized with enhanced chemiluminescence (GE Healthcare; RPN2106). To compare the amount of LAM normalized to the amount of His₆-tagged protein, two-color western analysis was performed using the Odyssey Western Analysis System (LI-COR, Lincoln, NB). Material was transferred to a nitrocellulose membrane and blocked with Odyssey blocking buffer (LI-COR, 927-40000) overnight at 4°C. Materials were detected by sequential incubations with antibodies diluted in a 50% solution of Odyssey blocking buffer in PBST. The membrane was incubated with mouse monoclonal anti-LAM (CS-35, NIAID HHSN266200400091C contract, Colorado State University, 1:50, overnight at 4°C), polyclonal goat anti-mouse (LI-COR, 926-32210, 1:10,000, for 2 h at 22°C), mouse monoclonal anti-His₆ (H-3, Santa Cruz, Santa Cruz, CA sc-8036; 1:1000, overnight at 4°C) and donkey anti-mouse (LI-COR, 926-32222; 1:10,000, for 2 h at 22°C).

Mass spectrometry and identification of ligands of NA-LprG

For nanospray mass spectrometry, 12 μg of purified protein was denatured in 500 μL methanol for more than 5 min, followed by vortexing. Ten μL of methanol eluate was loaded onto an in-house made glass nanospray tip for negative-mode electrospray ionization mass spectrometry (LCQ Advantage, Thermo Finnigan, Ringoes, NJ). For LC-MS analysis, 4.3 nanomoles of each protein was extracted in 500 μL methanol, dried under nitrogen, re-dissolved in 200 μL of HPLC mobile phase solution (95% A: 5% B, see below), and loaded on to a monochrome diol column (46 mm x 250 mm, 3 μm; Varian Inc., Palo Alto,
CA) and coupled on-line to a LXQ 2 dimensional ion-trap mass spectrometer (Thermo Finnigan) equipped with an electrospray ionization (ESI) source. The solvent system for mobile phase and gradient method for separation were slightly modified from a previous method. Briefly, mobile phase A was hexane:isopropanol (60:40, vol/vol) containing 0.1% (vol/vol) formic acid, 0.05% (vol/vol) ammonium hydroxide, and 0.05% (vol/vol) triethylamine. Mobile phase B was methanol containing 0.1% (vol/vol) formic acid, 0.05% (vol/vol) ammonium hydroxide, and 0.05% (vol/vol) triethylamine. At a flow rate of 0.7 ml/min, the binary gradient started at 5% mobile phase B, linearly increasing to 15% B in 6 min and then held at 15% B for 10 min, followed by linearly increasing to 95% B in 8 min, held at 95% mobile phase B for 6 min and finally adjusted back to 5% B in 2 min.

Expression and purification of NA-LprG for crystallography studies

Since analysis of LprG by secondary structure prediction and SignalP predicted unfavorable crystal packing with the first N-terminal 35 residues and the last C-terminal 5 residues, these residues were excluded from the portion of the LprG coding region that was amplified from Mtb H37Rv genomic DNA using the upstream primer 5’-

GGGAATTCCATATGCATCATCATCATCATCATGGACCACTTCCGG

ACGCGAAGCCGCTG -3’ (with an Ndel restriction site and His₆ tag) and the downstream primer 5’-

TCGACTCGAGCGTGACCTGGACCTTCTCGCCCCA

TTTCGA-3’ (with a Xhol restriction site). The amplified product and pET30b (Novagen) were digested with the restriction enzymes and ligated with T4 ligase
The pET30b-LprG plasmid was transformed into BL21 (DE3) (Novagen) and autotroph *E. coli* B834 (DE3) (Novagen). Transformants were plated on LB agar containing 50 µg/mL kanamycin. Cell cultures were grown in LB medium at 37°C to an *A*<sub>600</sub> of 0.6 and induced with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) at 16°C for 16 h. For seleno-methionine preparations, cells were grown in M9 minimal medium containing 19 amino acids but not methionine. Seleno-methionine (50mg/liter) and kanamycin (50 µg/mL) were added to cultures. Cell cultures were grown to A600 of 0.6 at 37°C and induced with 0.5 mM IPTG. After growth overnight at 16°C for 16 h, cells were pelleted and stored at -80°C.

For NA-LprG for crystallization, cell pellets were resuspended in buffer A (25 mM Tri-HCL pH 7.1, 500 mM NaCl, 2 mM β-mercaptoethanol) with DNase I (10 µg/ml) and 1 mM PMSF before lysis with 3 cycles of bead beater (Biospec). The lysate was centrifuged at 10,000 g for 60 min. The supernatant was loaded onto a Ni-column (GE Healthcare) and washed with 5 column volumes of buffer A. LprG bound to the Ni-column was eluted with an increasing gradient of buffer B (25 mM Tri-HCL pH 7.1, 500 mM NaCl, 500 mM imidazole, 2 mM β-mercaptoethanol). The fractions with LprG were pooled and dialyzed at 4°C against buffer D (20 mM Tris-HCl, pH 7.1, 150 mM imidazole, 2 mM β-mercaptoethanol).

**Crystallization of apo-NA-LprG, NA-LprG-PIM and NA-LprG-V91W**

Pure seleno-methionine (Se-Met) NA-LprG and native NA-LprG protein samples were pooled together. Apo-NA-LprG was concentrated to 20 mg/ml with an Amicon centrifugal filter (Millipore, 10,000 Dalton cut-off) and diluted with
water to 10 mg/mL for crystallization. A reducing agent, 0.5 mM Tris 2-carboxyethyl phosphine hydrochloride (TCEP), was added before setting plates. Mutant NA-LprG-V91W was diluted to 10 mg/ml. Initial screening for crystallization of NA-LprG, Se-Met NA-LprG and mutant NA-LprG-V91W was done using the sitting drop vapor diffusion method with commercially provided conditions from Crystal Screen, PEG Ion, Index screen and Salt RX (Hampton Research) and Wizard (Emerald Biostructures). The drop size was 4 μl in total with 2 μl of each protein solution and well solution. Se-Met crystals were obtained from 0.1 M Bis-Tris pH 6.5, 25% PEG3350. These crystals were optimized with the hanging drop method. NA-LprG crystals, obtained from 0.1 M sodium acetate trihydrate, pH 4.5, 25% PEG3350, were also optimized by the hanging drop method. NA-LprG-V91W crystals were produced in 0.2 M ammonium sulfate, 0.1 M sodium acetate tri-hydrate, pH 4.6, 30% PEG monomethyl ether 2000. For co-crystallization with glycolipids, 0.5 mM NA-LprG was incubated for one hour at room temperature with an equimolar mixture of PIM and phosphatidylinositol from *M. smegmatis*. The co-crystallization trial used the hanging drop vapor diffusion method. Extracted PIM/PI glycolipid mixtures from *M. smegmatis* and NA-LprG were incubated under the original hit conditions for native NA-LprG. Crystals were obtained with glycolipid mixture in 0.1 M sodium acetate trihydrate pH 4.5, 25% PEG3350.

*Data collection, data processing and structure determination of Se-Met NA-LprG, NA-LprG, NA-LprG-PIM bound and mutant NA-LprG-V91W*
Se-Met NA-LprG crystals were flash frozen in liquid nitrogen using 30% ethylene glycol as cryoprotectant, and structural data were collected in Lawrence Livermore National Laboratory. One 3-wavelength MAD data set was collected to resolution of 1.9 Å (Table 1). Native NA-LprG was collected to resolution of 2 Å with copper source and Raxis IV++ detector in lab (Rigaku). The data was processed with HKL2000. The space group of Se-Met NA-LprG was C2 and cell dimension was a= 95.5 Å, b= 72.3 Å, c= 62.3 Å, α=90°, β=106.8°, γ=90°. Native NA-LprG with space group C2 had cell dimension of a= 95.30 Å, b= 71.9 Å, c= 61.3 Å, α=90°, β=106.3°, γ=90°, which is close to Se-Met NA-LprG. Both had two molecules in an asymmetric unit. Phase was obtained from Se-Met MAD NA-LprG data set. AUTOSHARP program was used to build an initial model. The initial model from AUTOSHARP was entered into native NA-LprG refinement with CCP4 REFMAC and PHENIX. Coot was used to modify the model according to the electron density map. Further refinement and model fitting with Coot yielded a complete chain A structure, and the final dimer model was completed by superimposing chain A on chain B initial model. Molecule A is used to represent the structure of NA-LprG, since a loop region in molecule B between β1 and β2 was missing in the final structure. With added water by PHENIX, the final $R_{work}$ and $R_{free}$ were 22.9% and 27.8% with few outliers from the loop regions in Ramachandran plot.

Crystal structure data for NA-LprG with PIM bound using paratone as a cryo-protectant was collected at beamline 23ID-B in Argonne National Lab to a resolution of 1.8 Å (Table 2). HKL2000 was used for data processing. Apo-NA-
LprG structure was applied and run rigid body refinement with PHENIX. Structures of ligand were generated by PHENIX and CCP4. Ligand was fitted with coot and refined with PHENIX. The final $R_{work}$ and $R_{free}$ were 22.2% and 25.8%. NA-LprG-V91W crystals using paratone as a cryo-protectant were collected at 19ID in Argonne National Lab. High resolution data was collected to 1.85 Å (Table 2) and processed with HKL2000 in P21 space group with cell dimension ($a= 39.72$ Å, $b= 56.0$ Å, $c= 96.9$ Å, $\alpha=90^\circ$, $\beta=99.6^\circ$, $\gamma=90^\circ$). Structural solution of the dimer asymmetric unit was obtained by molecular replacement method. Structure of NA-LprG-V91W was refined with PHENIX, under modification with COOT. The final $R_{work}$ and $R_{free}$ were 21.5% and 25.3%.

Protein structure graphics were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (NIH P41 RR-01081; 11). Predicted structures of LprA and the fusions between LprA and LprG were generated using CPH model v2.0 (http://www.cbs.dtu.dk/services/CPHmodels/) and MODELLER (http://www.salilab.org/modeller). Cavity sizes of proteins were calculated by using CastP program (http://sts.bioengr.uic.edu/castp/calculation) with spherical probe of 1.4 Å 12.

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