# SUPPORTING INFORMATION

# Alanyl-Phosphatidylglycerol Synthase: Mechanism of Substrate Recognition during tRNA-dependent Lipid Modification in *Pseudomonas aeruginosa*

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# Supplemental Table S1

TABLE S1. **Enzymatic activity of A-PGS$_{543-881}$ after chemical modification or treatment with chelating agents under *in vitro* and modified *in vitro* (using Ala-tRNA$^{Ala}$) conditions.**

A-PGS was purified in the absence of reducing agents and subsequently modified with phenylmethylsulfonyl fluoride, diisopropylfluorophosphate and p-hydroxyphenylglyoxal, respectively. After an extensive dialysis step proteins were subjected to the standard *in vitro* and modified *in vitro* A-PGS$_{543-881}$ assays as described under "Experimental Procedures". Ethylenediaminetetraacetic acid and 1,10-phenanthroline, respectively, were added directly to the assays. The relative *in vitro* activity and activity in modified *in vitro* assays of the corresponding unmodified A-PGS$_{543-881}$ was set as 100 %, and all other values were related to this value. Assays were performed in duplicate, standard deviation ± 10 %. n.t. = not tested.

| **Modifying / chelating agent** | **Concentration [mM]** | **Relative activity in *in vitro* assays [%]** | **Relative activity in modified *in vitro* assays [%]** |
|---|---|---|---|
| phenylmethylsulfonyl fluoride | 0.1 | 100 | n.t. |
| | 1 | 100 | n.t. |
| diisopropylfluorophosphate | 0.1 | 100 | n.t. |
| | 1 | 100 | n.t. |
| p-hydroxyphenylglyoxal | 0.5 | 90 | n.t. |
| | 1 | 90 | 100 |
| | 5 | 60 | n.t. |
| | 10 | 60 | 100 |
| ethylenediaminetetraacetic acid | 3 | 100 | 100 |
| | 10 | 100 | 100 |
| | 20 | n.t. | 100 |
| 1,10-phenanthroline | 1 | 100 | n.t. |
| | 3 | n.t. | 100 |
| | 5 | n.t. | 75 |
| | 10 | 45 | 35 |
| | 20 | n.t. | 20 |

# Supplemental Table S2

TABLE S2. **Determination of metal ion content of purified A-PGS$_{543-881}$.**

Purified GST-A-PGS$_{543-881}$ protein was concentrated (10.5 mg/ml – 161 µM) and subjected to inductively coupled plasma - mass spectrometry (ICP-MS) experiments. In parallel the sole GST protein (4 mg/ml – 154 µM) was analyzed to determine the background level of the employed purification strategy. Experiments were performed in duplicate.

| **Metal ion** | **Concentration of metal ion in GST-A-PGS$_{543-881}$ [µM]** | **Concentration of metal ion in GST [µM]** |
|---|---|---|
| $Fe^{2+}$ | 4.3 | 1.6 |
| $Ni^{2+}$ | 0.2 | 0.2 |
| $Cu^{2+}$ | 0.3 | 0.2 |
| $Mg^{2+}$ | 18.8 | 1.2 |
| $Mn^{2+}$ | 0.4 | 0.02 |
| $Zn^{2+}$ | 0.9 | 0.2 |

# Supplemental Table S3

TABLE S3. **Primers used in this study.**
The sequence for T7 promoter is underlined; restriction sites are shown in italics. Exchanged nucleotides for mutagenesis of *P. aeruginosa* A-PGS are in bold.

| Primer | | |
|---|---|---|
| 1 | 5pGPaBamf | GTA*GGATCC*CGCGCGGCACC |
| 2 | 6pGPaXhor | GAT*CTCGAG*TCAGCGTTTCACCAATC |
| 3 | QCA856NSTOPfw | CTACCTGGCCGTGCCA**AACTA**GGGCTCGACCCGCTGGTG |
| 4 | QCA856NSTOPrv | CACCAGCGGGTCGAGCCC**TAGTT**TGGCACGGCCAGGTAG |
| 5 | 27alaSNdeIfw | ATAGC*CATATGA*AAAGCGCTGAAATCCG |
| 6 | 28alaSBclIrv | ATAGC*TGATCA*TCAGAGCCCTTGCTCGAC |
| 7 | D579Afw | GCCCTGACCGGCG**CT**AAGGCCCTGCTC |
| 8 | D579Arv | GAGCAGGGCCTT**AG**CGCCGGTCAGGGC |
| 9 | D579Nfw | GCCCTGACCGGC**A**ACAAGGCCCTGCTC |
| 10 | D579Nrv | GAGCAGGGCCTTGT**T**GCCGGTCAGGGC |
| 11 | K580Sfw | CCCTGACCGGCGAC**TCT**GCCCTGCTCTTCC |
| 12 | K580Srev | GGAAGAGCAGGGC**AGA**GTCGCCGGTCAGGG |
| 13 | K580Qfw | CTGACCGGCGAC**C**AGGCCCTGCTCTTC |
| 14 | K580Qrev | GAAGAGCAGGGCCTGGTCGCCGGTCAG |
| 15 | Y635Afw | CCCGCCCGGTGTTC**GCT**CAGGTGCGTGCCG |
| 16 | Y635Arev | CGGCACGCACCTG**AGC**GAACACCGGGCGGG |
| 17 | K654Sfw | GGCCTGACCGCCCTC**TCT**CTCGGCGAAGAAGC |
| 18 | K654Srev | GCTTCTTCGCCGAG**AGA**GAGGGCGGTCAGGCC |
| 19 | K654Qfw | CTGACCGCCCTC**C**AGCTCGGCGAAGAAG |
| 20 | K654Qrev | CTTCTTCGCCGAGCT**G**GAGGGCGGTCAG |
| 21 | E657Dfw | CTCAAGCTCGGCGA**C**GAAGCGCGAGTC |
| 22 | E657Drv | GACTCGCGCTTC**G**TCGCCGAGCTTGAG |
| 23 | E657Qfw | CCTCAAGCTCGGC**C**A**G**GAAGCGCGAGTCG |
| 24 | E657Qrv | CGACTCGCGCTTC**C**T**G**GCCGAGCTTGAGG |
| 25 | E658Dfw | CAAGCTCGGCGAAGA**C**GCGCGAGTCGAC |
| 26 | E658Drv | GTCGACTCGCGC**G**TCTTCGCCGAGCTTG |
| 27 | E658Qfw | CTCAAGCTCGGCGAA**C**A**G**GCGCGAGTCGACC |
| 28 | E658Qrv | GGTCGACTCGCGC**C**T**G**TTCGCCGAGCTTGAG |
| 29 | S709Afw | GAGCTGAAGGCGATA**G**C**T**GACGCCTGGCTG |
| 30 | S709Arv | CAGCCAGGCGTC**A**G**C**TATCGCCTTCAGCTC |
| 31 | S709Nfw | GAGCTGAAGGCGATA**AAC**GACGCCTGGCTGGGC |
| 32 | S709Nrv | GCCCAGCCAGGCGTC**GTT**TATCGCCTTCAGCTC |
| 33 | D710Afw | GAAGGCGATATCGG**CT**GCCTGGCTGGGCGG |
| 34 | D710Arv | CCGCCCAGCCAGGC**AG**CCGATATCGCCTTC |
| 35 | D710Nfw | CTGAAGGCGATATCG**A**ACGCCTGGCTGGGC |
| 36 | D710Nrv | GCCCAGCCAGGCGT**T**CGATATCGCCTTCAG |
| 37 | E720Qfw | GCAAGCAGGTGCGC**C**AGAAAGGCTTCTCCC |
| 38 | E720Qrv | GGGAGAAGCCTTTCT**G**GCGCACCTGCTTGC |
| 39 | S724Afw | CGCGAGAAAGGCTTC**G**C**T**CTGGGCCGCTTCAC |

| | | |
|---|---|---|
| 40 | S724Arv | GTGAAGCGGCCCAG**A**G**C**GAAGCCTTTCTCGCG |
| 41 | S724Nfw | CGCGAGAAAGGCTTC**AA**CCTGGGCCGCTTCACC |
| 42 | S724Nrv | GGTGAAGCGGCCCAGG**TT**GAAGCCTTTCTCGCG |
| 43 | Y732Afw | CCGCTTCACCCCGGCG**GCT**CTGAACTTCTTCCGC |
| 44 | Y732Arev | GCGGAAGAAGTTCAG**AGC**CGCCGGGGTGAAGCGG |
| 45 | S763Afw | GCCGCGAACTGGCG**GCT**CTCGACCTGATGCGC |
| 46 | S763Arv | GCGCATCAGGTCGAG**AGC**CGCCAGTTCGCGGC |
| 47 | S763Nfw | CGCGAACTGGCGA**A**CCTCGACCTGATGC |
| 48 | S763Nrv | GCATCAGGTCGAGG**T**TCGCCAGTTCGCG |
| 49 | D765Afw | GAACTGGCGAGCCTCG**CT**CTGATGCGCGTG |
| 50 | D765Arev | CACGCGCATCAG**AG**CGAGGCTCGCCAGTTC |
| 51 | D765Nfw | CTGGCGAGCCTC**A**ACCTGATGCGCG |
| 52 | D765Nrev | CGCGCATCAGG**T**TGAGGCTCGCCAG |
| 53 | R768Sfw | GAGCCTCGACCTGATG**TCT**GTGCACCCGGACGCG |
| 54 | R768Srev | CGCGTCCGGGTGCAC**AGA**CATCAGGTCGAGGCTC |
| 55 | R768Qfw | CCTCGACCTGATGC**AG**GTGCACCCGGACG |
| 56 | R768Qrev | CGTCCGGGTGCAC**CT**GCATCAGGTCGAGG |
| 57 | M778Afw | CGCGCCGAAGCTGACC**GCT**GAGTTCCTCATGCTC |
| 58 | M778Arev | GAGCATGAGGAACTC**AGC**GGTCAGCTTCGGCGCG |
| 59 | Y831Afw | GCGGGGCGAACAGTTC**GCT**AATTTCCAGGGGCTGC |
| 60 | Y831Arev | GCAGCCCCTGGAAATT**AGC**GAACTGTTCGCCCCGC |
| 61 | R837Sfw | CTACAATTTCCAGGGGCTG**TCT**CGCTTCAAGGACAAGTTCC |
| 62 | R837Srev | GGAACTTGTCCTTGAAGCG**AGA**CAGCCCCTGGAAATTGTAG |
| 63 | R837Qfw | CTACAATTTCCAGGGGCTG**CAG**CGCTTCAAGGACAAGTTC |
| 64 | R837Qrev | GAACTTGTCCTTGAAGCG**CTG**CAGCCCCTGGAAATTGTAG |
| 65 | K840Sfw | CAGGGGCTGCGACGCTTC**TCT**GACAAGTTCCAGCCC |
| 66 | K840Srev | GGGCTGGAACTTGTC**AGA**GAAGCGTCGCAGCCCCTG |
| 67 | K840Qfw | GCTGCGACGCTTC**C**AGGACAAGTTCCAGC |
| 68 | K840Qrev | GCTGGAACTTGTCCT**G**GAAGCGTCGCAGC |
| 69 | K842Sfw | CTGCGACGCTTCAAGGAC**TCT**TTCCAGCCCGACTGGG |
| 70 | K842Srev | CCCAGTCGGGCTGGAA**AGA**GTCCTTGAAGCGTCGCAG |
| 71 | K842Qfw | GACGCTTCAAGGAC**C**AGTTCCAGCCCGAC |
| 72 | K842Qrev | GTCGGGCTGGAACT**G**GTCCTTGAAGCGTC |
| 73 | alatRNA1fw | *AATTC*TAATACGACTCACTATAGGGGCCATAGCTCAGCTGGGAGAGCGCCTG CTTTGCACGCAGGAGGTCAGGAGTTCGATCCTCCTTGGCTCCACCA*G* |
| 74 | alatRNA1rv | *GATCC*TGGTGGAGCCAAGGAGGATCGAACTCCTGACCTCCTGCGTGCAAAG CAGGCGCTCTCCCAGCTGAGCTATGGCCCCTATAGTGAGTCGTATTA*G* |
| 75 | alatRNA2fw | *AATTC*TAATACGACTCACTATAGGGGCTATAGCTCAGCTGGGAGAGCGCTTG CATGGCATGCAAGAGGTCGACGGTTCGATCCCGTCTAGCTCCACCA*G* |
| 76 | alatRNA2rv | *GATCC*TGGTGGAGCTAGACGGGATCGAACCGTCGACCTCTTGCATGCCATGC AAGCGCTCTCCCAGCTGAGCTATAGCCCCTATAGTGAGTCGTATTA*G* |

# Supplemental Table S4

TABLE S4. ***E. coli*** **strains and plasmids used in this study.**

| **Bacterial strain or plasmid** | | **Genotype or phenotype** | **Reference** |
|---|---|---|---|
| *E. coli* | | | |
| | BL21 (λDE3) | B $F^-$ *dcm ompT hsdS*($r_B^-$ $m_B^-$) *gal* λ(DE3) | Stratagene, La Jolla, USA |
| | Rosetta (DE3) pLysS | $F^-$ *ompT hsdS*$_B$($r_B^-$ $m_B^-$) *gal dcm* (DE3) pLysSRARE$^2$ ($Cam^r$) | Novagen, Darmstadt, Germany |
| | TOP10 | F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔ M15 Δ*lac*X74 *rec*A1 *ara* Δ139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L ($Str^r$) *end*A1 *nup*G | Invitrogen (Grant *et al.*, 1990) |
| | K-12, strain AG1, clone JW2667 | *recA1 endA1 gyrA96 thi-1 hsdR17(*$rK^-$ $mK^+$*) supE44 relA1* carrying plasmid pCA24N which encodes for AlaRS from *E. coli*, $Cam^r$ | (Kitagawa *et al.*, 2005) |
| | K-12, strain AG1, clone JW2498 | *recA1 endA1 gyrA96 thi-1 hsdR17(*$rK^-$ $mK^+$*) supE44 relA1* carrying plasmid pCA24N which encodes for HisRS from *E. coli*, $Cam^r$ | (Kitagawa *et al.*, 2005) |
| Plasmids | | | |
| | pBADmyc-His-A/ PA0920 | pBADmyc-His-A containing PA0920 gene fused to the sequence for C-terminal $His_6$-tag cloned between *Hind*III and *Nco*I sites, $Ap^r$ | (Klein *et al.*, 2009) |
| | pBAD-His-A/ PA0920 | pBAD-His-A containing the PA0920 gene fused to the sequence for N-terminal $His_6$-tag cloned between *Xho*I and *Hind*III sites, $Ap^r$ | (Klein *et al.*, 2009) |
| | pBAD-His-A/ $PA0920_{1\text{-}855N}$ | pBAD-His-A/PA0920-derivative, deletion of amino acids 857 – 881, amino acid exchange alanine 856 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 | Expression vector for *E. coli* carrying base pairs 1627 - 2643 of ORF PA0920 cloned into *Bam*HI/*Xho*I sites, *tac*-promoter, sequence for an N-terminal GST-tag with specific restriction site for PreScission™ Protease, $Ap^r$ | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D579A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 579 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D579N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 579 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K580S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 580 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K580Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 580 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 Y635A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange tyrosine 635 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K654S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 654 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K654Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 654 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 E657D | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange glutamate 657 to aspartate | This work |

| | | | |
|---|---|---|---|
| | pGEX-6P-1/ PA0920Δaa1-542 E657Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange glutamate 657 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 E658D | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange glutamate 658 to aspartate | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 E658Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange glutamate 658 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S709A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 709 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S709N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 709 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D710A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 710 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D710N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 710 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 E720Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange glutamate 720 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S724A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 724 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S724N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 724 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 Y732A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange tyrosine 732 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S763A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 763 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 S763N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange serine 763 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D765A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 765 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 D765N | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange aspartate 765 to asparagine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 R768S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange arginine 768 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 R768Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange arginine 768 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 M778A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange methionin 768 to alanine | This work |

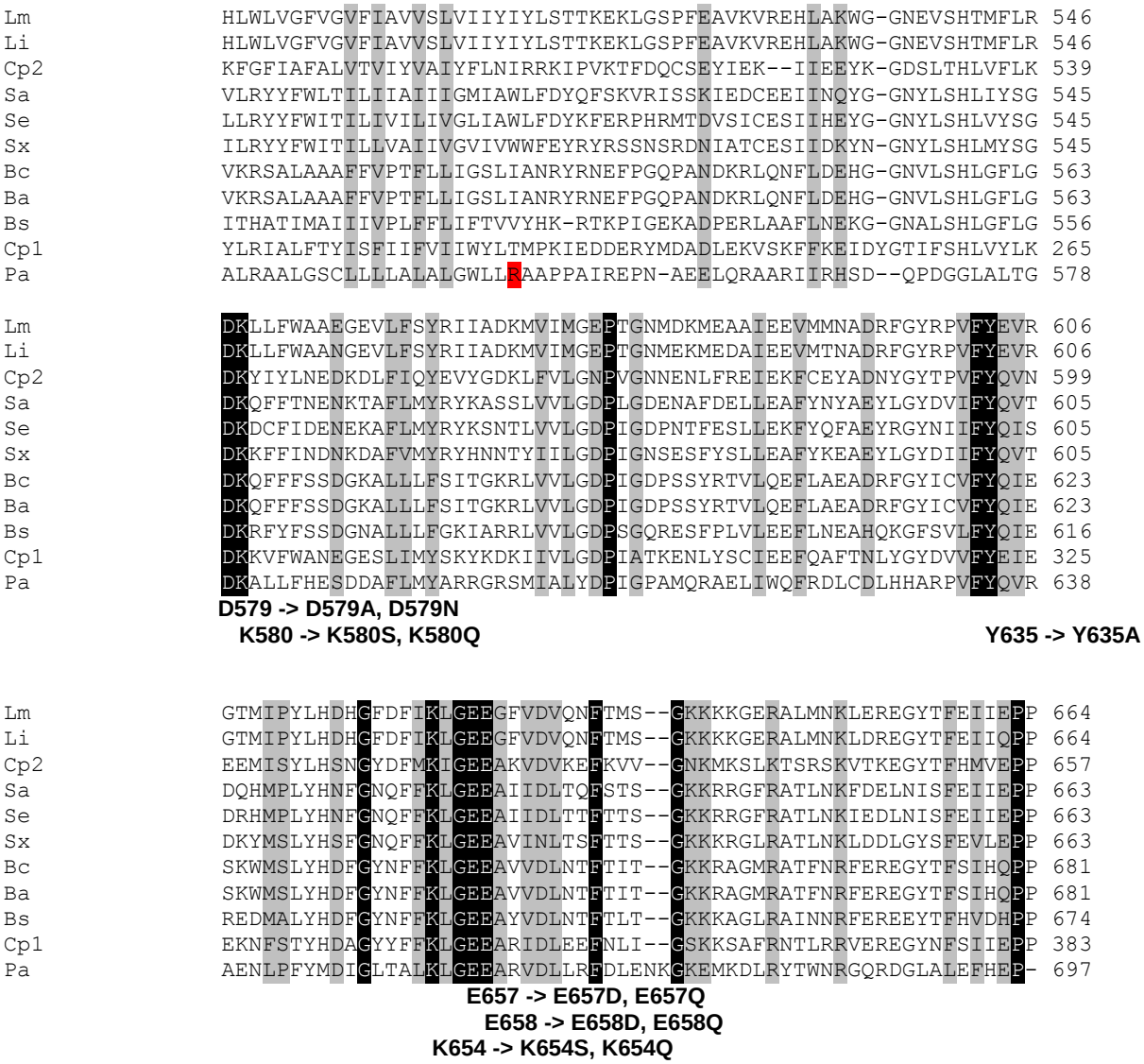
| | | | |
|---|---|---|---|
| | pGEX-6P-1/ PA0920Δaa1-542 Y831A | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange tyrosine 831 to alanine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 R837S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange arginine 837 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 R837Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange arginine 837 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K840S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 840 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K840Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 840 to glutamine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K842S | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 842 to serine | This work |
| | pGEX-6P-1/ PA0920Δaa1-542 K842Q | pGEX-6P-1/PA0920Δaa1-542-derivative, amino acid exchange lysine 842 to glutamine | This work |
| | pGEX-6P-1/ A-PGS$_{543\text{-}855N}$ | pGEX-6P-1/PA0920Δaa1-542-derivative, deletion of amino acids 857 – 881, amino acid exchange alanine 856 to asparagine | This work |
| | pET28b(+)PA0903 | Expression vector for *E. coli* carrying the gene encoding alanyl-tRNA-synthetase (ORF PA0903) cloned into *Nde*I/*Bam*HI sites, T7-promoter, sequence for an N-terminal His-tag, Kan$^r$ | This work |
| | pUC18tRNA$^{Ala1}$ | pUC18 containing ORF PA4280.3 (coding for tRNA$^{Ala1}$) from *P. aeruginosa* cloned into the *Eco*RI and *Bam*HI restriction sites of the MCS under control of a T7 promoter and with a 3' *Bst*NI restriction site, Ap$^r$ | This work |
| | pUC119tRNA$^{Ala2}$ | pUC119 containing ORF PA3133.2 (coding for tRNA$^{Ala2}$) from *P. aeruginosa* cloned into the *Eco*RI and *Bam*HI restriction sites of the MCS under control of a T7 promoter and with a 3' *Bst*NI restriction site, Ap$^r$ | This work |

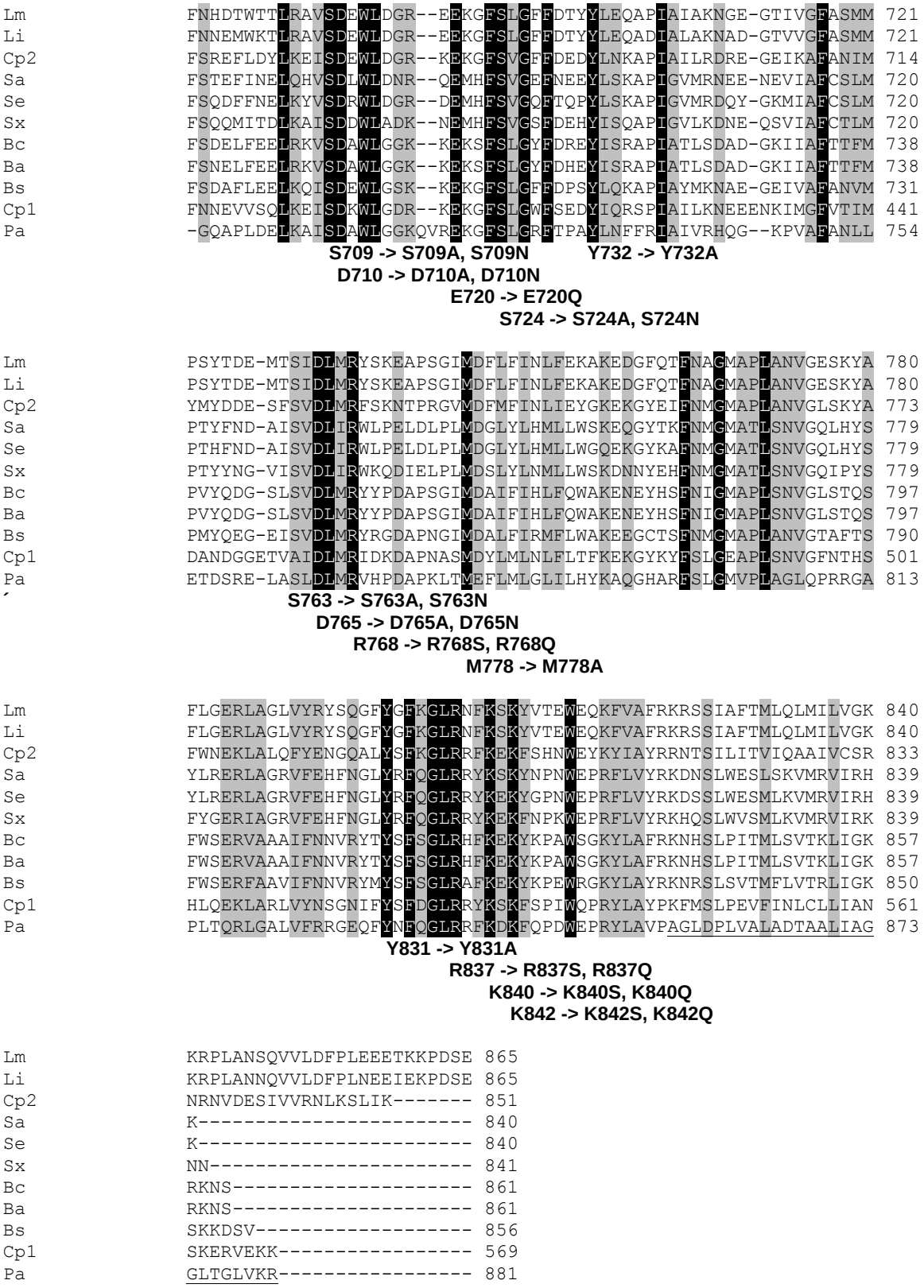
# Supplemental Figure S1

FIG. S1. **Sequence alignment of orthologous aa-PGS proteins.**

Homologous aa-PGS proteins from *Listeria monocytogenes* (Lm), *Listeria innocua* (Li), *Clostridium perfringens* MprF2 (Cp2), *Staphylococcus aureus* (Sa), *Staphylococcus epidermis* (Se), *Staphylococcus xylosus* (Sx), *Bacillus cereus* (Bc), *Bacillus anthracis* (Ba), *Bacillus subtilis* (Bs), *Clostridium perfringens* MprF1 (Cp1) and *Pseudomonas aeruginosa* (Pa). Partially conserved positions are boxed in gray, completely conserved positions in black. The first amino acid of the soluble A-PGS$_{543-881}$ variant from *P. aeruginosa* is highlighted in red. The proposed C-terminal helix is underlined. Amino acid positions that have been mutagenized by site-directed mutagenesis and point mutations that have been analyzed in the present study are indicated.

## CLUSTAL 2.0.12 multiple sequence alignment

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Li FNNEMWKTLRAVSDEWLDGR--EEKGFSLGFFDTYYLEQADIALAKNAD-GTVVGFASMM 721
Cp2 FSREFLDYLKEISDEWLDGR--KEKGFSVGFFDEDYLNKAPIAILRDRE-GEIKAFANIM 714
Sa FSTEFINELQHVSDLWLDNR--QEMHFSVGEFNEEYLSKAPIGVMRNEE-NEVIAFCSLM 720
Se FSQDFFNELKYVSDRWLDGR--DEMHFSVGQFTQPYLSKAPIGVMRDQY-GKMIAFCSLM 720
Sx FSQQMITDLKAISDDWLADK--NEMHFSVGSFDEHYISQAPIGVLKDNE-QSVIAFCTLM 720
Bc FSDELFEELRKVSDAWLGGK--KEKSFSLGYFDREYISRAPIATLSDAD-GKIIAFTTFM 738
Ba FSNELFEELRKVSDAWLGGK--KEKSFSLGYFDHEYISRAPIATLSDAD-GKIIAFTTFM 738
Bs FSDAFLEELKQISDEWLGSK--KEKGFSLGFFDPSYLQKAPIAYMKNAE-GEIVAFANVM 731
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Pa -GQAPLDELKAISDAWLGGKQVREKGFSLGRFTPAYLNFFRIAIVRHQG--KPVAFANLL 754
S709 -> S709A, S709N
Y732 -> Y732A
D710 -> D710A, D710N
E720 -> E720Q
S724 -> S724A, S724N
Lm PSYTDE-MTSIDLMRYSKEAPSGIMDFLFINLFEKAKEDGFQTFNAGMAPLANVGESKYA 780
Li PSYTDE-MTSIDLMRYSKEAPSGIMDFLFINLFEKAKEDGFQTFNAGMAPLANVGESKYA 780
Cp2 YMYDDE-SFSVDLMRFSKNTPRGVMDFMFINLIEYGKEKGYEIFNMGMAPLANVGLSKYA 773
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Se PTHFND-AISVDLIRWLPELDLPLMDGLYLHMLLWGQEKGYKAFNMGMATLSNVGQLHYS 779
Sx PTYYNG-VISVDLIRWKQDIELPLMDSLYLNMLLWSKDNNYEHFNMGMATLSNVGQIPYS 779
Bc PVYQDG-SLSVDLMRYYPDAPSGIMDAIFIHLFQWAKENEYHSFNIGMAPLSNVGLSTQS 797
Ba PVYQDG-SLSVDLMRYYPDAPSGIMDAIFIHLFQWAKENEYHSFNIGMAPLSNVGLSTQS 797
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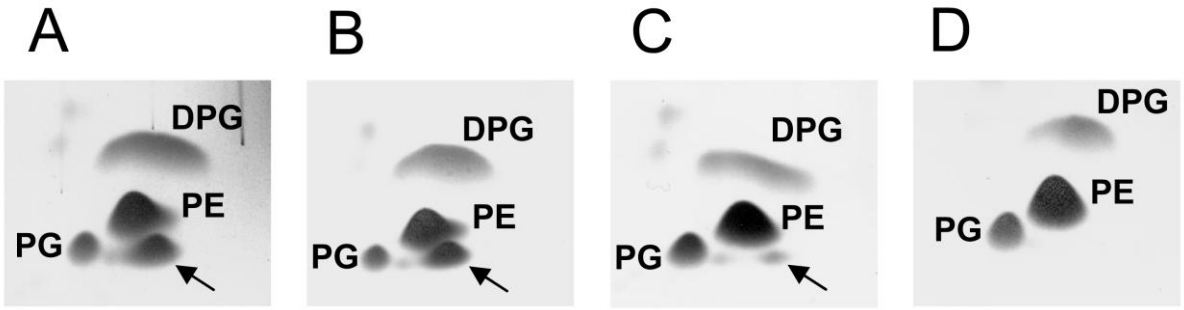
Pa ETDSRE-LASLDLMRVHPDAPKLTMEFLMLGLILHYKAQGHARFSLGMVPLAGLQPRRGA 813
S763 -> S763A, S763N
D765 -> D765A, D765N
R768 -> R768S, R768Q
M778 -> M778A
Lm FLGERLAGLVYRYSQGFYGFKGLRNFKSKYVTEWEQKFVAFRKRSSIAFTMLQLMILVGK 840
Li FLGERLAGLVYRYSQGFYGFKGLRNFKSKYVTEWEQKFVAFRKRSSIAFTMLQLMILVGK 840
Cp2 FWNEKLALQFYENGQALYSFKGLRRFKEKFSHNWEYKYIAYRRNTSILITVIQAAIVCSR 833
Sa YLRERLAGRVFEHFNGLYRFQGLRRYKSKYNPNWEPRFLVYRKDNSLWESLSKVMRVIRH 839
Se YLRERLAGRVFEHFNGLYRFQGLRRYKEKYGPNWEPRFLVYRKDSSLWESMLKVMRVIRH 839
Sx FYGERIAGRVFEHFNGLYRFQGLRRYKEKFNPKWEPRFLVYRKHQSLWVSMLKVMRVIRK 839
Bc FWSERVAAAIFNNVRYTYSFSGLRHFKEKYKPAWSGKYLAFRKNHSLPITMLSVTKLIGK 857
Ba FWSERVAAAIFNNVRYTYSFSGLRHFKEKYKPAWSGKYLAFRKNHSLPITMLSVTKLIGK 857
Bs FWSERFAAVIFNNVRYMYSFSGLRAFKEKYKPEWRGKYLAYRKNRSLSVTMFLVTRLIGK 850
Cp1 HLQEKLARLVYNSGNIFYSFDGLRRYKSKFSPIWQPRYLAYPKFMSLPEVFINLCLLIAN 561
Pa PLTQRLGALVFRRGEQFYNFQGLRRFKDKFQPDWEPRYLAVPAGLDPLVALADTAALIAG 873
Y831 -> Y831A
R837 -> R837S, R837Q
K840 -> K840S, K840Q
K842 -> K842S, K842Q
Lm KRPLANSQVVLDFPLEEETKKPDSE 865
Li KRPLANNQVVLDFPLNEEIEKPDSE 865
Cp2 NRNVDESIVVRNLKSLIK------- 851
Sa K------------------------ 840
Se K------------------------ 840
Sx NN---------------------- 841
Bc RKNS-------------------- 861
Ba RKNS-------------------- 861
Bs SKKDSV------------------ 856
Cp1 SKERVEKK---------------- 569
Pa GLTGLVKR---------------- 881

## Supplemental Figure S2

FIG. S2. **Activity of mutant A-PGS proteins under *in vivo* conditions.**

Separation of polar membrane lipids by 2D-TLC and detection by 5% (w/v) molybdatophosphoric acid staining is shown. Lipids were extracted from *E. coli* BL21 (λDE3) cells overproducing different A-$PGS_{543-881}$ mutants. Positions of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) are shown. A-PG is indicated by an arrow.

A-PG formation by the wild type A-$PGS_{543-881}$ protein is shown in *panel A*. A-PG formation by the mutant enzymes in the *in vivo* assay were classified as: +++, activity comparable to wild type enzyme (mutant protein K654S, *panel B*); +, decreased activity when compared to wild type enzyme (mutant protein Y732A, *panel C*); -, no detectable A-PGS activity (mutant protein D765N, *panel D*).




### References Supporting Information

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