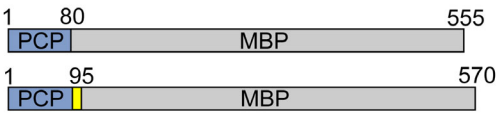
Supporting Information

(A)



(B)

N-terminal PCP	GFP	GST	MBP	β-Gal	Luc
PCP^{EntB}	1	5	9	13	17
PCP^{GrsA}	2	6	10	14	18
PCP^{EntB}-$(SGGGG)_3$-	3	7	11	15	19
PCP^{GrsA}-$(SGGGG)_3$-	4	8	12	16	20

Figure S1. (A) Schematic representation of PCP^{GrsA} fused N-terminal to MBP with and without linker (yellow) in between PCP and MBP. The numbers denote the site of the fusion and the length of the protein. (B) Numbering of the PCP fusions constructed in this study. "β-Gal" denotes β-galactosidase and "Luc" denotes luciferase.

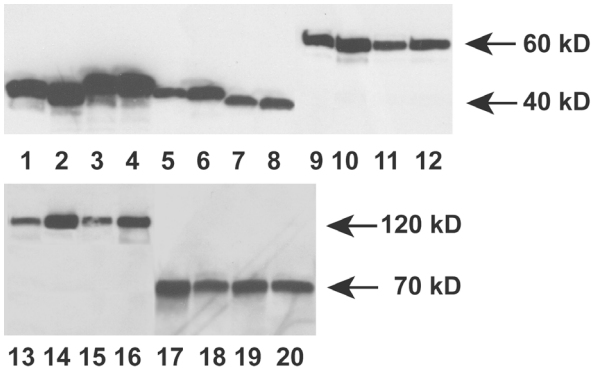


Figure S2. Western blotting analysis of the biotin labeling reaction carried out with the cell lysates. The blots were probed with streptavidin HRP conjugate and the lane numbering is the same as shown in Figure S1 (B).

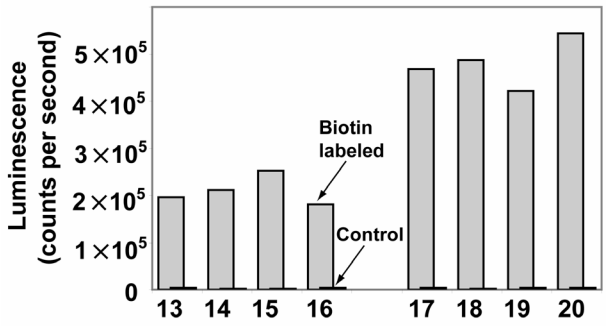


Figure S3. Assay of β-galactosidase (13-16) and luciferase (17-20) activity immobilized on the streptavidin plates. The gray bars represent the enzymatic activities of the wells with biotin labeled cell lysates added. Control reactions were run with biotin CoA **1** replaced by acetyl CoA. The lane numbering is the same as shown in Figure S1 (B).

Synthesis of biotin CoA 1.

The synthesis of Biotin CoA **1** was previously reported in reference 7e. To a solution of biotin maleimide (Pierce) (10 mg, 0.019 mmol) in 300 μL DMSO, coenzyme A lithium salt (Sigma) (18.2 mg, 0.023 mmol) in 2 ml MES acetate 50 mM, pH 6.0 was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then purified by preparative HPLC on a reversed-phase C18 column with a gradient of 0-60% acetonitrile in 0.1% TFA/water over 35 minutes. The purified compound was lyophilized and the identity was confirmed by LC-MS operating in the positive ion mode.

Construction of the fusion protein genes.

Blow is the procedure for the construction of plasmids pET-PCP^{EntB}-GtfB and pET-PCP^{GrsA}-GtfB for the expression of PCP – glycosyltransferase GtfB fusions in conjunction with our studies on GtfB (unpublished results). These plasmids were then used as the templates for the assembly of the genes of the PCP fusion proteins reported in this study.

The gene of PCP^{EntB} was amplified from plasmid pFR1[8a] with primers Jun31 (5'- TAA GAA GGA GAT ATA CAT ATG TCC CTG) and Jun32 (5'- CGA TCC GCC ACC GCC AGA GCC ACC TCC GCC TGA ACC GCC TCC ACC TGA AAG CTT CAC CTC GCG GGA GAG). The gene of PCP^{GrsA} was amplified from plasmid pPheATE[8b] with primers Jun38 (5'- GAG GAG AAA TTA CAT ATG GCG GAA CCT GAT) and Jun39 (5'- CGA TCC GCC ACC GCC AGA GCC ACC TCC GCC TGA ACC GCC TCC ACC TGA AAG CTT ACT ATC TTT TAT ATA TTG). Jun31 and Jun38 introduced a NdeI restriction site at the 5'end of the PCR fragments and Jun32 and Jun39 introduced a HindIII restriction site at the 3' end of the PCR fragments. GtfB gene was amplified from plasmid pET22b-gtfB (Losey, H. C. *et al*, Biochemistry, 2001, 40, 4745-4755) with primers Jun33 (5'- TCT GGC GGT GGC GGA TCG GCT AGC CGT GTG CTG TTG GCG ACG) and Jun35 (5'- AGC AGC CGG ATC TCA GTG G). Jun33 introduced a NheI restriction site at the 5'- end of the GtfB gene and a stretch of 18 base overlap with the 3' end of the amplified PCP^{EntB} and PCP^{GrsA} gene. The PCP gene fragments and the GtfB gene fragment were then assembled to the gene of N-terminal PCP – GtfB fusion by overlap extension PCR with the following conditions in a 50 μL total volume: 5 μL 10× Herculase Enhanced DNA polymerase buffer (Stratagene), 0.25 mM each dNTPs (Biorad), 0.25 μM each primers, 0.2 μg each PCR amplified DNA fragments, 5% DMSO and 1 unit Herculase Enhanced DNA polymerase (Stratagene). PCR fragments of PCP^{EntB} was assembled with GtfB with primers Jun31 and Jun35 and PCR fragments of PCP^{GrsA} was assembled with GtfB with primers Jun38 and Jun35. Amplification was carried out with a Robocycler (Stratagene) at 94°C for 45 seconds, 50°C for 1 minute and 72 °C for 3.5 minutes for 30 cycles. The assembled PCR fragments were purified by 1% agarose gel electrophoresis and the bands of the desire size were cut out from the gel and DNA eluted from the gel by electrophoresis. By overlap extension, a stretch of 15 residue linker $(Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_3$ was inserted in

between PCP and GtfB flanked by a 5'- HindIII and a 3'- NheI restriction site. The assembled DNA fragments were digested by restriction enzymes NdeI and XhoI and cloned into the pET22b vector (Novagen) with an ampicillin resistant marker and a His_6 tag attached to the C-terminal of the fusion protein resulting in the plasmids pET-PCP^{EntB}-GtfB and pET-PCP^{GrsA}-GtfB.

EGFP gene was amplified from plasmid pEGFP (Clontech, BD Biosciences) with primers Jun55a (5'- GTA CCG GTC GCC AAG CTT GTG AGC AAG GGC GAG G) and Jun57 (5'- AGA GTC GCG GCC CTC GAG CTT GTA CAG CTC GTC C) or Jun56 (5'- GTA CCG GTC GCC GCT AGC GTG AGC AAG GGC GAG G) and Jun57. GST gene was amplified from plasmid pGEX-4T-1 (Amersham Pharmacia) with primers Jun58 (5'- GAA ACA GTA TTC AAG CTT CCT ATA CTA GGT TAT TGG) and Ju60 (5'- GGA TCC ACG CGG CTC GAG ATC CGA TTT TGG) or with Jun59 (5'- GAA ACA GTA TTC GCT AGC CCT ATA CTA GGT TAT TGG) and Jun60. MBP gene was amplified from plasmid pMal-c2x (Biorad) with primers Jun61 (5'- GGA CCA TAG CAT AAG CTT ATC GAA GAA GGT AAA CTG G) and Jun63 (5'- CAG GTC GAC TCT CTC GAG TCC GAA TTC TGA AAT CC) or with primers Jun62 (5'- GGA CCA TAG CAT GCT AGC ATC GAA GAA GGT AAA CTG G) and Jun63. LacZ gene encoding β-galactosidase was amplified from plasmid pβgal-Basic (Clontech, BD Biosciences) with primers Jun64 (5'- CTG CTC AAG CGC AAG CTT GTC GTT TTA CAA CGT CG) and Jun66 (5'- CCT GCC CGG TTA CTC GAG TTT TTG ACA CCA GAC C) or with primers Jun65 (5'- CTG CTC AAG CGC GCT AGC GTC GTT TTA CAA CGT CG) and Jun66. Luciferase gene was amplified from plasmid pBI-L (Clontech, BD Biosciences) with primers Jun67 (5'- GCA GCC CGT ACC AAG CTT GAC GCC AAA AAC ATA AAG) and Jun69 (5'- GAA TAC AGT TAC CTC GAG CAA TTT GGA CTT TCC) or with primers Jun68 (5'- GCA GCC CGT ACC GCT AGC GAC GCC AAA AAC ATA AAG) and Jun69. Primers Jun55a, Jun58, Jun61, Jun64 and Jun67 introduced a HindIII site at the 5' end of each PCR fragment. Primers Jun56, Jun59, Jun62, Jun65 and Jun68 introduced a NheI site at the 5' end of each PCR fragment. Primers Jun57, Jun60, Jun63, Jun66 and Jun69 introduced a XhoI site at the 3' end of each PCR fragment. The PCR fragments were cloned into the pET-PCP-GtfB plasmids preciously constructed using the restriction sites HindIII and XhoI to give the direct fusion of PCP^{EntB} or PCP^{GrsA} with EGFP, GST, MBP, β-galactosidase and luciferase, respectively. When the PCR fragments were cloned into the plasmid pET-PCP-GtfB with the NheI and XhoI restriction sites, fusion protein genes were constructed with linker $(Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_3$ inserted in between PCP and EGFP, GST, MBP, β-galactosidase and luciferase, respectively.

Fusion protein expression and purification.

The pET vectors containing the genes of the C-terminal His_6 tagged fusion proteins were transformed into *E. coli* BL21(DE3)pLysS chemical competent cells (Invitrogen). Cells were grown at 37°C in Luria-Bertani (LB) media supplemented with 100 mg/mL ampicillin to an OD_{600} of 0.5. Then the temperature was reduced to 20°C, 1 mM isopropyl-D-thiogalactopyranoside (IPTG) added, and the culture grown overnight. The cells were harvested by centrifugation (4000*g*, 15 min) and the pellets were

resuspended in the lysis buffer (50 mM Tris, 0.5 M NaCl, 5 mM imidazole, pH 8.0) with 1 unit/ml DNAse I and disrupted by French Press (Thermo Spectronic) with two passes at 16 000 psi. Cell debris was removed by ultracentrifugation (95000*g*, 30 min). The clarified cell extract was incubated with Ni-NTA resin (Qiagen; ~1 mL of 50% suspension/L of cell culture) for 3-4 h at 4°C in a batch-binding format. The suspension was then loaded into a gravity column and washed with lysis buffer. Proteins bounded to the column were eluted with 250 mM imidazole in buffer A. The purity of the fractions containing the purified protein of interest was checked by SDS-PAGE with Coomassie staining. Fractions with the desired purity were pooled and dialyzed against 2 × 1 L of 50 mM HEPES, 100 mM NaCl, 10% glycerol, pH 7.5. Protein solutions were then stored at -80°C at a concentration higher than 5 mg/mL.

Protein expression in the 96 well plate.

High throughput expression of the fusion proteins and subsequent cell lysis were performed following the procedure in reference 10. Briefly, 1 mL LB supplemented with 100 mg/mL ampicillin was distributed to each well of a 2 mL deep well 96 well assay block. Each well was then inoculated with 10 μL overnight starting culture. The plate was then shaken at 250 rpm at 37°C for 8 hours before the addition of 10 μL 20 mM IPTG to each well to induce fusion protein expression. The temperature was then lowered to 20°C and the cultures were allowed to grow overnight. The next morning, 100 μL 10× PBS supplemented with 10 unit/mL DNase I (Sigma) and 100 μL 10 mg/mL deoxycholic acid were added to each well of the cell culture and the plate was incubated at 20°C for another 4 hours with shaking at 250 rpm. The cell lysate was then ready for biotin CoA **1** labeling.

Biotin CoA labeling reaction.

Sfp catalyzed biotin CoA labeling of the purified fusion proteins were performed following the procedure described in reference 7c. In a total volume of 100 μL, 0.5 μM Sfp phosphopantetheinyl transferase, 5 μM biotin CoA **1**, 10 mM $MgCl_2$ and 1 mM tris(2-carboxyethyl)phosphine (TCEP) in 50 mM HEPES pH 7.5 were incubated with 20 μM fusion protein for 30 minutes. The labeling reaction mixtures were then assayed by Western blotting and enzyme linker immune assays (ELISA). For the labeling reaction with the cell lysate from high throughput protein expression, to each well of the standard 96 well plate was added 90 μL labeling reaction mixture with the same composition described above without the purified fusion protein. Then 10 μL cell lysate was transferred from each well of the deep well assay block to the labeling reaction mixture in the shallow well plate. The labeling reaction in the standard 96 well plate was incubated at 30°C for 30 minutes and biotin labeling was assayed by ELISA or Western blotting. For both biotin labeling reactions of the purified protein and the cell lystate, control reactions were run with same concentration of acetyl CoA in stead of biotin CoA.

^{3}H acetyl CoA chase.

After incubating at 30°C for 30 minutes, 20 μL of the cell lysate biotin labeling reaction from the previous step was added 4 μL of ^{3}H acetyl CoA (American Radiolabeled Chemicals) (418 μM, 200 μCi/mol) and incubated at 30°C for another 30 minutes. The reaction was then quenched by addition of 200 μL 10% (w/v) trichloroacetic acid (TCA) and centrifuged at 4°C for 6 minutes at 13,000 rpm. The supernatant was discarded and the pellet was washed twice with 1 ml TCA each time. Finally the pellet was redissolved in 180 μL 88% formic acid, added to 5 ml scintillation fluid and counted for ^{3}H radioactivity on a LS 6500 multipurpose scintillation counter (Beckman Coulter). In the control reaction, biotin CoA was eliminated from the labeling reaction and followed the exact same procedure. The ^{3}H radioactivity precipitated by TCA was then compared for the biotin labeling reaction and the control reaction.

ELISA and Western blotting.

Streptavidin coated 96 well plate (Pierce) was first blocked with 3% BSA in TBS (25 mM TrisHCl, pH 7.5 and 100 mM NaCl) by incubating with the BSA solution at room temperature for 1 hour. The blocking solution was then removed and to each well was added 90 μL 1% BSA in TBS buffer. 10 μL loading reaction mixture was than added to each well and cross-plate 5 fold dilution was performed. The control reaction with acetyl CoA added instead of **1** was also added to the streptavidin plate for comparison. After incubation at room temperature for one hour, the plate was washed five times with 0.05% Tween 20 and 0.05% Triton X-100 in TBS and five times with TBS. 100 μL 1% BSA containing a mixture of mouse anti-GFP, anti-GST and anti-MBP antibodies (Santa Cruz Biotechnology) was added to each well and incubated for 1 hour at room temperature. The plate was again washed five times with 0.05% Tween 20 and 0.05% Triton X-100 in TBS and five times with TBS. Then 100 μL 1/5000 dilution of 1 mg/mL goat anti-mouse IgG antibody -horse radish peroxidase (HRP) conjugate (Pierce) was added to each well and incubated at room temperature for another hour followed by washing the plate with 0.05% Tween 20 and 0.05% Triton X-100 in TBS for five times and TBS for five times. Finally the bound peroxidase activity of each well was detected by TMB substrate kit. (Pierce).

For western blotting, 10 μL labeling reaction mixture was loaded on a 4-15% SDS-PAGE gel (Biorad). After electrophoresis, the protein bands were electroblotted onto a piece of PVDF membrane (Biorad). The membrane was then blocked with 3% BSA in TBS for 2 hours followed by incubation with $1/10^{6}$ diluted 1 mg/mL streptavidin - HRP conjugate (Pierce) in 1% BSA for an hour. The membrane was then washed with 0.05% Tween 20 and 0.05% Triton X-100 in TBS for five times followed by detection with the ECL luminescent detection kit (Amersham Pharmacia).

Antibody - fluorophore conjugation.

The anti-GST and anti-MBP antibodies (Santa Cruz Biotechnology) were labeled with Alexa Fluor 488 and Alexa Fluor 647 amine reactive dyes (Molecular Probes), respectively. In each case, the antibody in PBS buffer was first concentrated to a concentration of 1 mg/ml. To an aliquot of 100 μL of the concentrated antibody solution

was added 10 μL of 1 M freshly prepared sodium bicarbonate solution, followed by the addition of the reactive dye solution provided by the manufacturer. The reaction was incubated for one hour in the dark with gentle shaking. The resulting labeled antibody was loaded onto a gel filtration column (30 kD MWCO) and spun at 1100 x g for five minutes. The purification procedure was repeated if the excess dye was not completely removed as judged by UV-Vis spectroscopy. Labeled anti-GST and anti-MBP displayed maxima of absorption at 495 nm and 650 nm, respectively. The anti-GFP antibody was purchased from Santa Cruz Biotechnology as TRITC conjugate.

Microarray spotting.

Avidin coated glass slides were prepared following the procedure reported in reference 3c. Epoxide functionalized glass slides (Corning) were coated with avidin and the epoxide surface was quenched with aspartic acid solution. The glass slides were further blocked by soaking in 3% BSA in TBS solution for overnight at 4°C before use. 20 μL labeling reaction mixtures containing biotin CoA **1** or acetyl CoA labeled cell lysates were transferred to a 384 well plate and spotted on to the avidin glass slide using GeneMachines OminiGrid microarray spotter. After spotting, the glass slides were washed three times in TBS with 0.05% Tween 20 and 0.05% Triton X-100, each time for 10 minutes. The glass slides were then dried under a nitrogen stream and 100 μL solution of 1-5 μg/mL of mixed anti-GFP, anti-GST and anti-MBP antibodies conjugated to fluorophores in 1% BSA TBS was laid on each glass slide and the slides were covered with cover slips. The slides were then incubated in hybridization cassettes (Corning) at room temperature for one hour before the glass slides were washed three times with 0.05% Tween 20 and 0.05% Triton X-100 in TBS, each time for 10 minutes. After wash, the slides were dried under nitrogen stream and scanned with a ScanArray Express microarray scanner (Packard Bioscience).

β-Galactosidase and luciferase assay.

Similar to the ELISA assay, the labeling reaction mixtures with β-galactosidase or luciferase PCP fusion proteins expressed were added to a white wall streptavidin coated 96 well plate (Pierce) to allow the binding between the biotin labeled enzymes with the streptavidin surface. The plates were then washed as in the ELISA assay and the enzymatic activity of each well was detected with β-galactosidase and luciferase detection kit (Clontech, BD Biosciences).