Supporting Information

General Utilization of Fluorescent Polyisoprenoids with Sugar Selective Phosphoglycosyltransferases.

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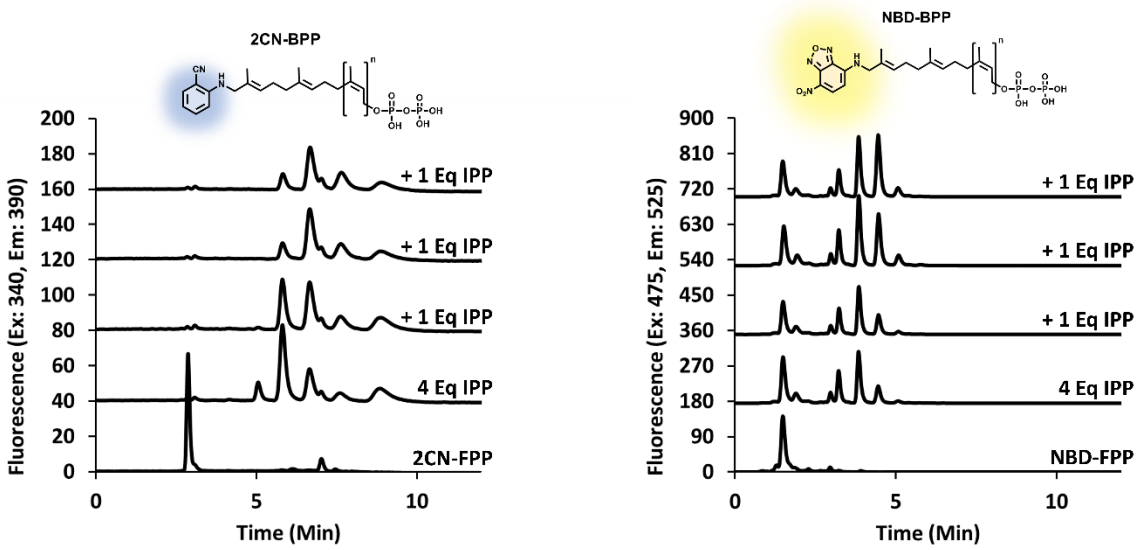


Figure S1. Tunable size of fl-BPPs. Representative UppS reactions were set up starting with 4 equivalents of IPP. Sequential additions of one equivalent isopentenyl diphosphate increases the size fl-BPP, while maintaining a relatively narrow distribution of BPP sizes.

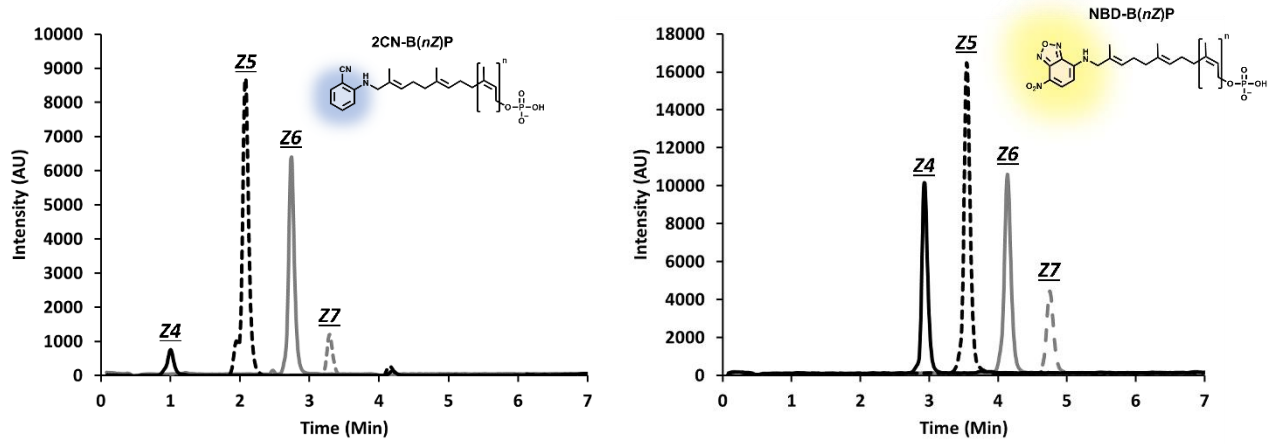


Figure S2. LCMS detection of fl-BPs. Variable length fl-BP's are observed in SIM mode on LCMS. *n*-Propanol was increased at a rate of 4% per min, and started at either 40% for 2CN or 20% for NBD with 60% and 80% 100 mM ammonium hydroxide, respectively, as the co-solvent. Mass values for *Z3-Z8* fl-BPs that were scanned for are in Table 1. Signals for fl-BP were approximately 10-fold lower than for fl-BPP of the same size. Thus, products were generally analyzed by LCMS after monophosphate following acid phosphatase reactions. Alternatively, fl-BPP products could be detected using the corresponding fl-BP value for *in situ* degradation to the monophosphate during ionization.

Table S1. SIM m/z values for fl-BPs. All presented values are the $[M-H]^-$ species for each size.

	m/z	
nZ	**2CN**	**NBD**
3	554.4	615.3
4	622.4	683.4
5	690.5	751.4
6	758.5	819.5
7	826.6	887.6
8	894.6	955.6

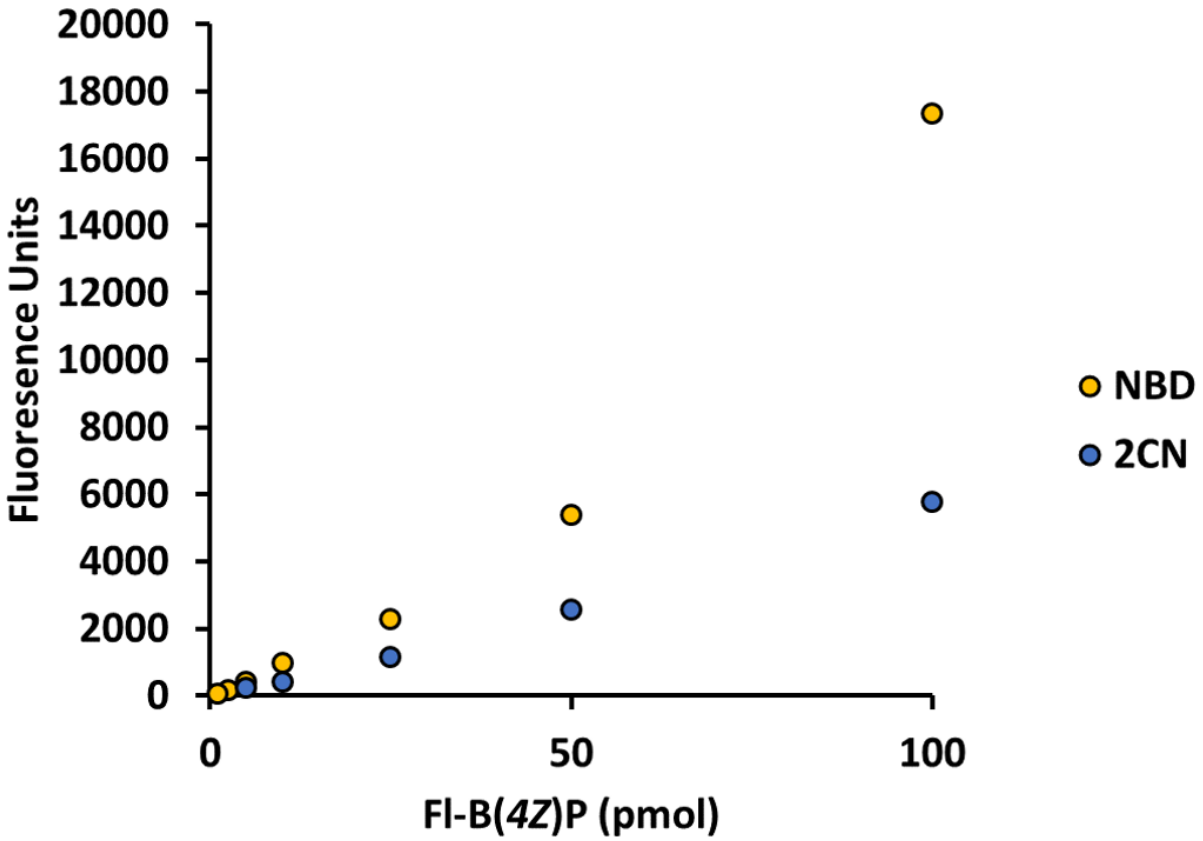


Figure S3. Limit of detection for fluorescent probes. Fluorescence units include area under the curve of fl-B(*4Z*)P of 2CN and NBD. Limit of detection is defined by the concentration at which the minimum detectable concentration is observed. For 2CN and NBD the limit of detection corresponded to 5 and 1 pmol, respectively.

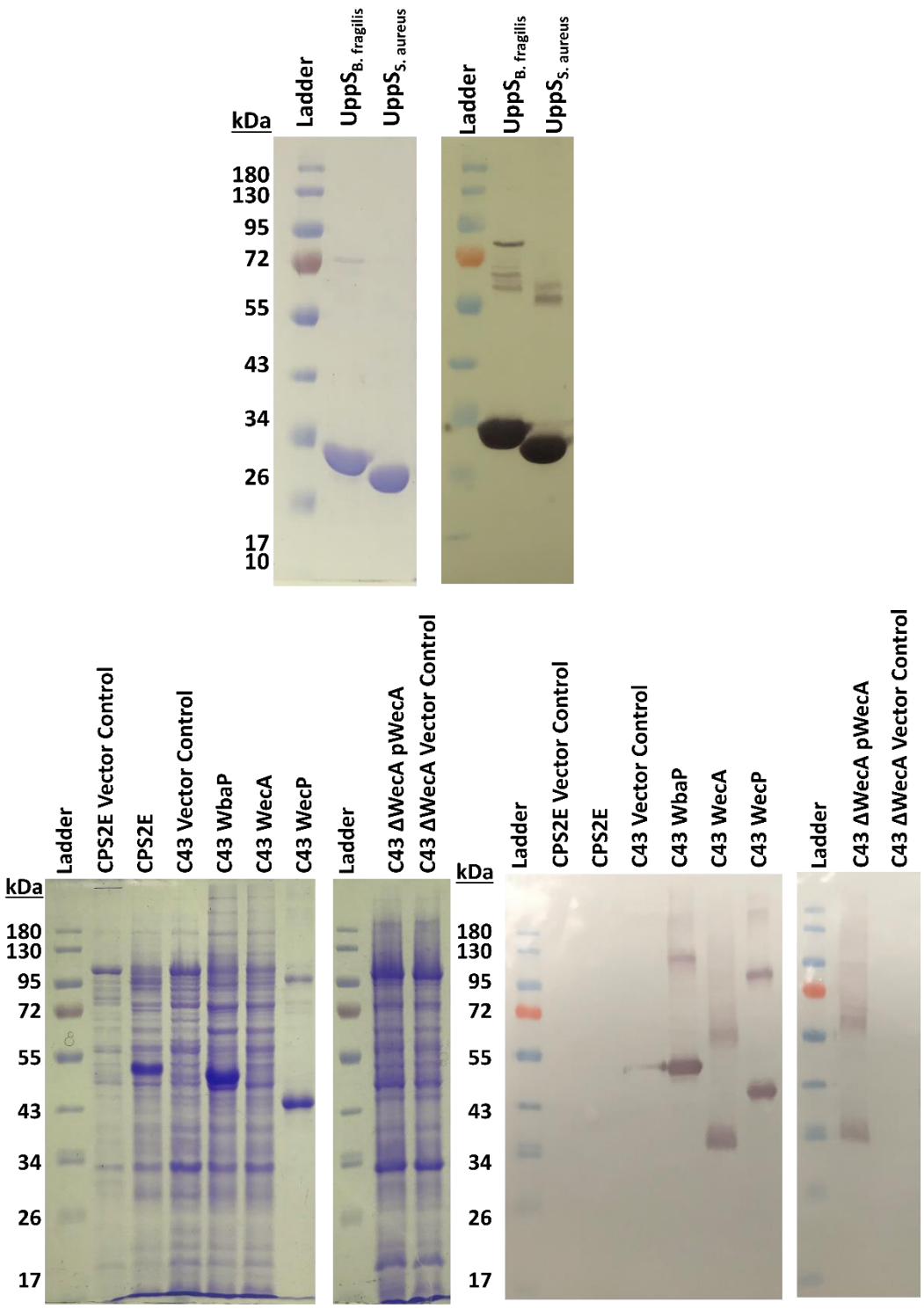


Figure S4. SDS-PAGE and corresponding western blot. SDS-PAGE stained with coomassie. All western blots were treated first with primary rabbit anti-His antibody (1:5,000 dilution), followed by secondary goat anti-rabbit antibody conjugated to alkaline phosphatasse (1:20,000 dilution). Staining occurred with a 1-Step BCIP/NBT substrate solution for <5 min.

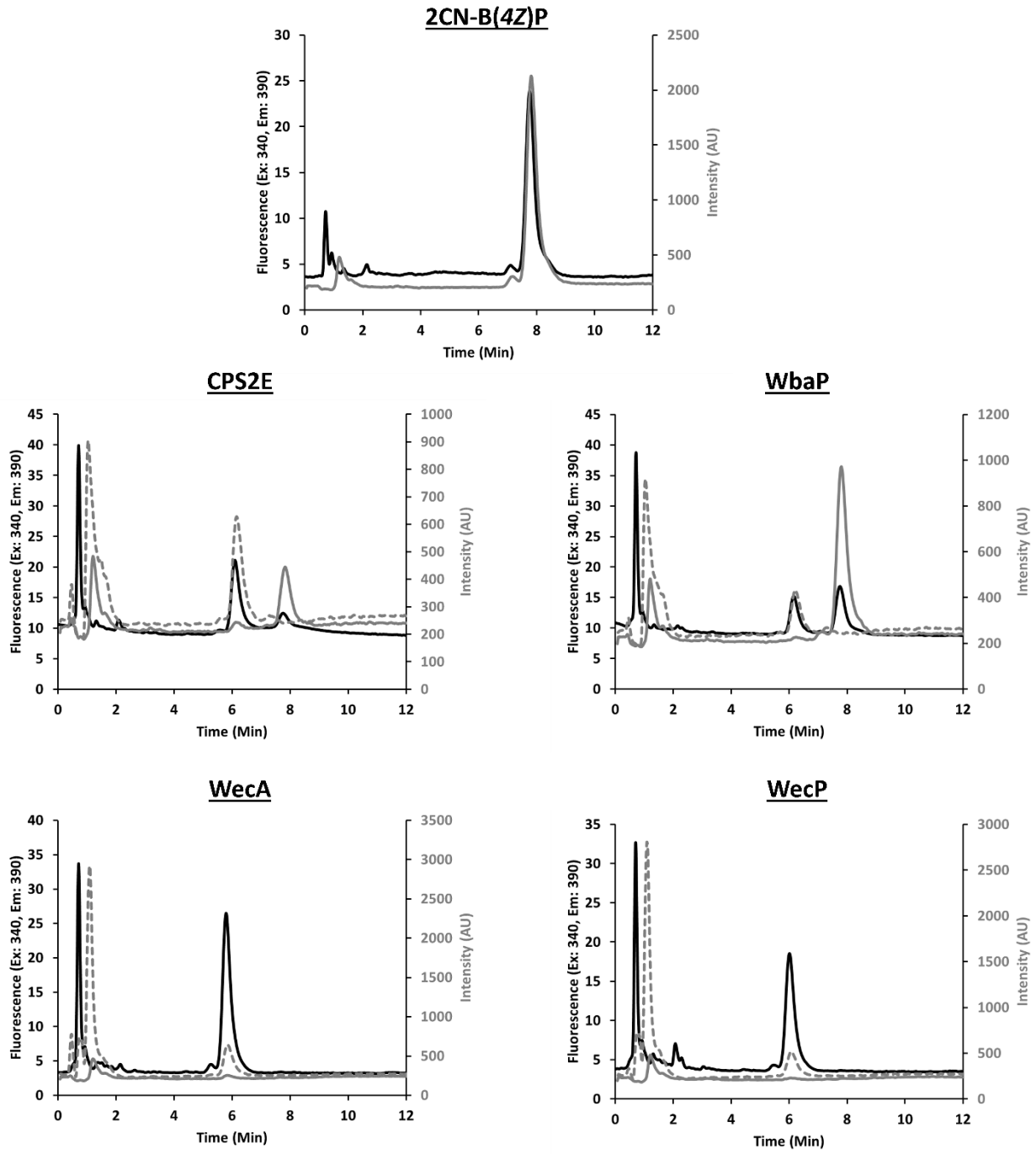


Figure S5. LCMS characterization of PGT activity with 2CN. LCMS analysis was carried out at 26% propanol isocratic with a TEE connector to the fluorescence detector (left axis) and mass spec of respective masses on SIM mode (right axis). The 2CN-B(*4Z*)P standard appears at a retention time of 7.8 min, with a corresponding $[M-H]^-$ solid gray line (621.4 m/z). 2CN-BPP-Hexose and 2CN-BPP-HexNAc both appear at a retention time of 6.0 min and an overlapping $[M-H]^-$ in dashed gray line (863.4 and 904.4 m/z, respectively).

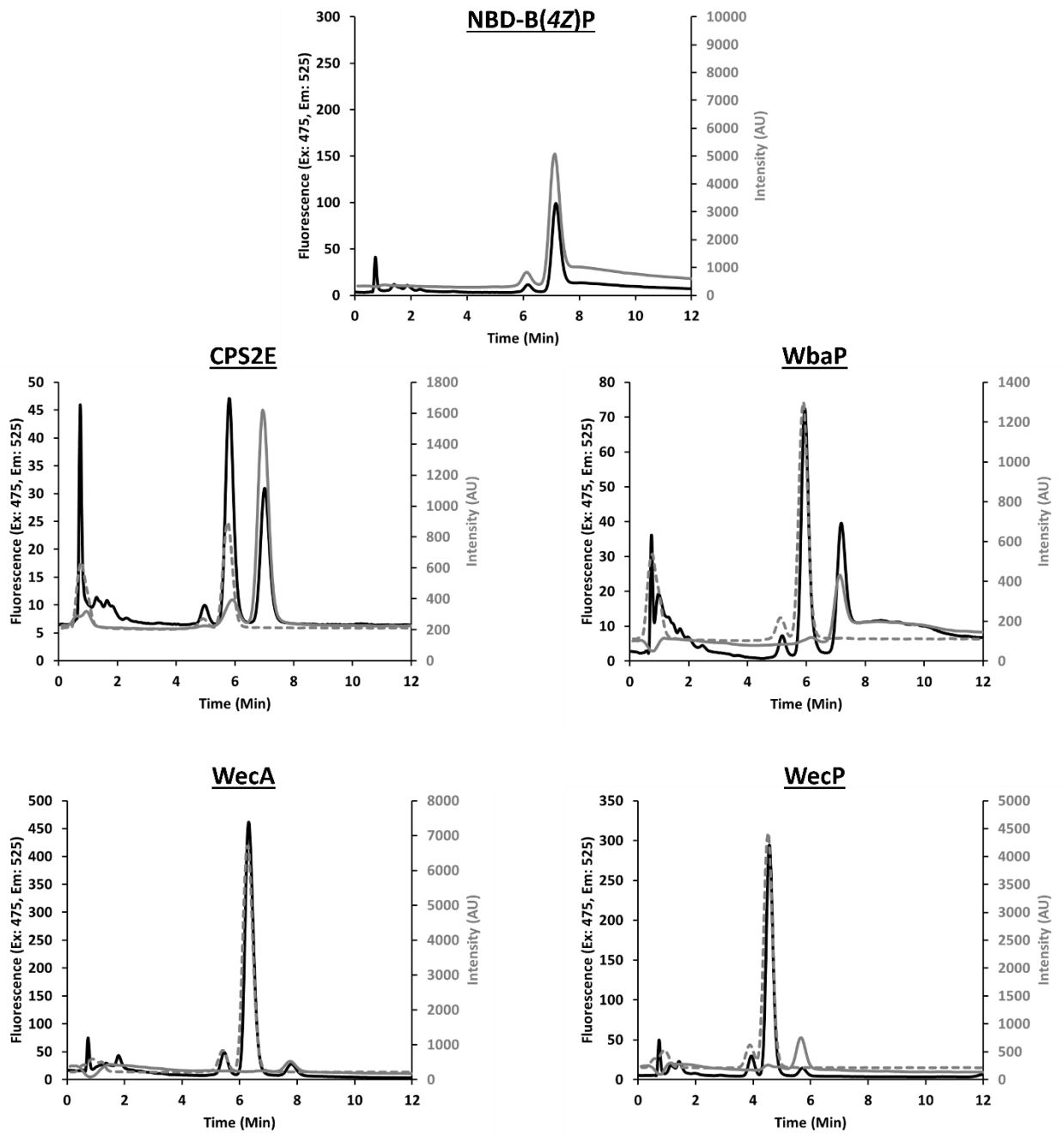


Figure S6. LCMS characterization of PGT activity with NBD. LCMS analysis was carried out at 24% propanol isocratic as described in Figure S5. The NBD-B(*4Z*)P standard appears at a retention time of 7.1 min, with a corresponding $[M-H]^-$ in solid gray line (683.4 m/z). NBD-BPP-Hexose and NDB-BPP-HexNAc both appear at a retention time of 5.8 min and an overlapping $[M-H]^-$ in dashed gray line (925.4 and 966.4 m/z, respectively).

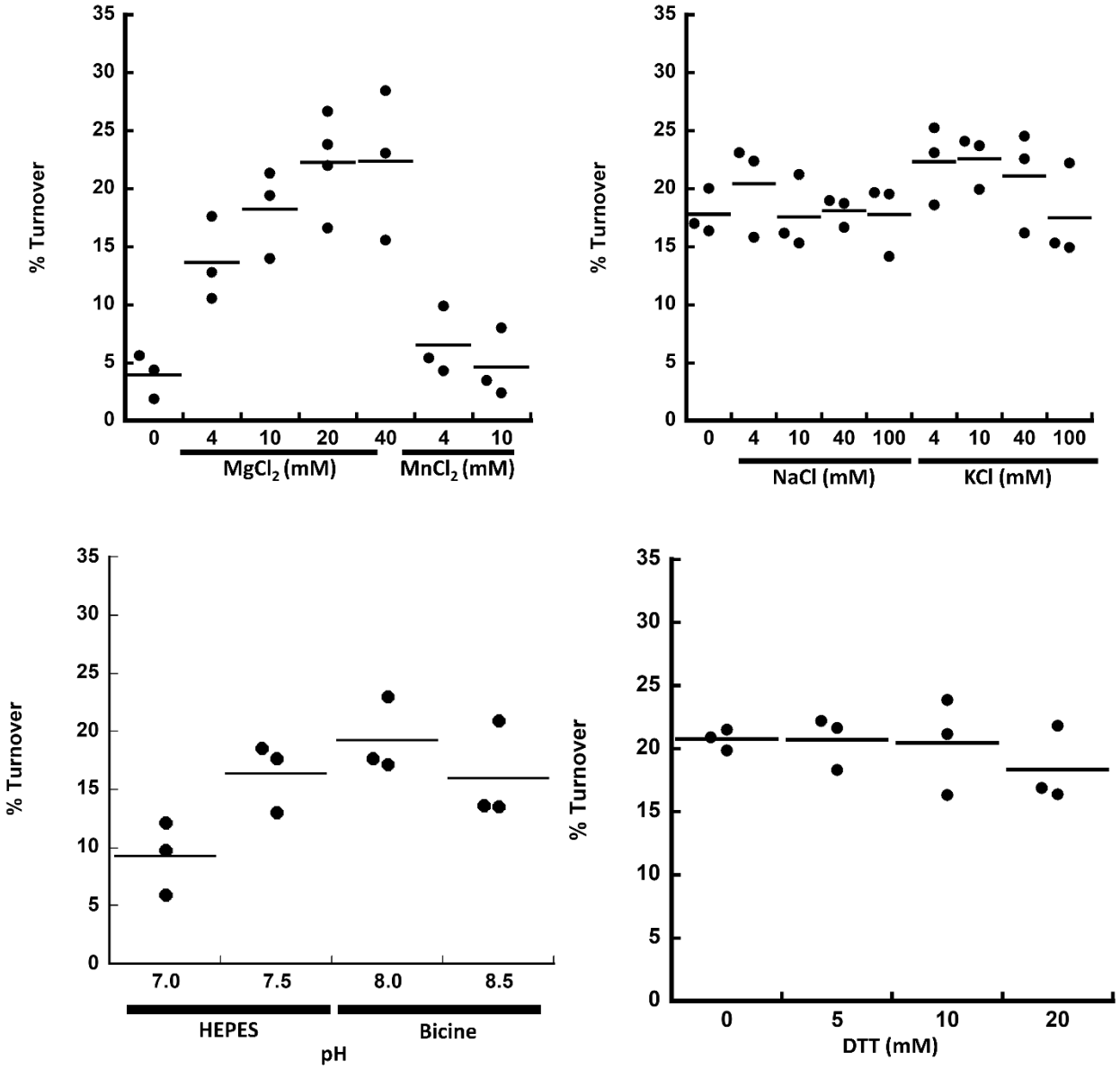


Figure S7. Optimization of WecP: Divalent cations, buffers, and DTT. When not varied, standard conditions included the following: 10 mM $MgCl_2$, 10 mM KCl, 200 mM Bicine (pH 8.0), 4 uM 2CN-B(*7Z*)P with 2% n-propanol, 216 uM UDP-GlcNAc, 20 mM DTT, 2.08 ug/mL WecP, and 0.0192% (2.4 xCMC) DDM. Reactions were quenched after 20 minutes and analyzed by fluorescence HPLC for total product turnover.

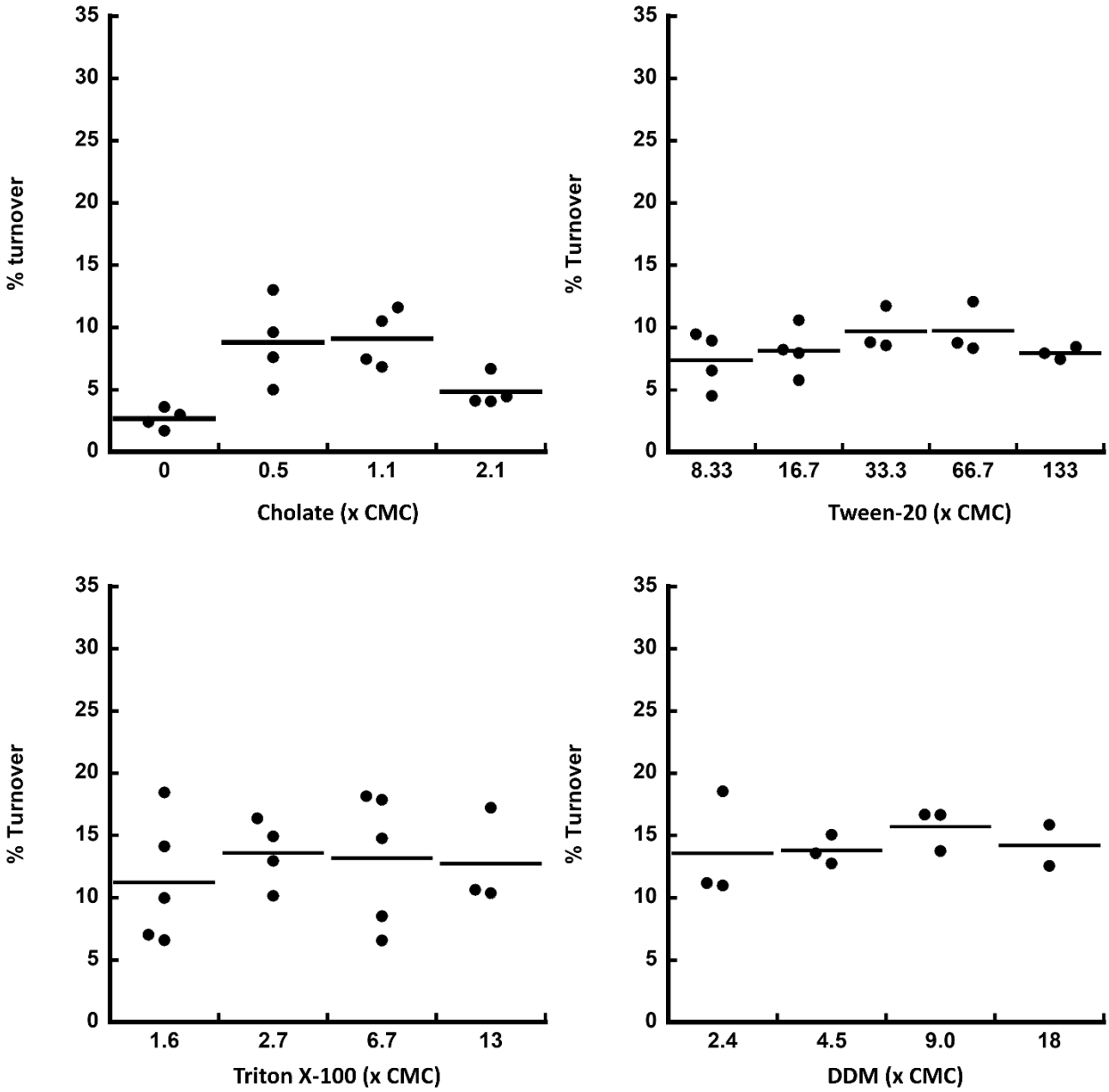
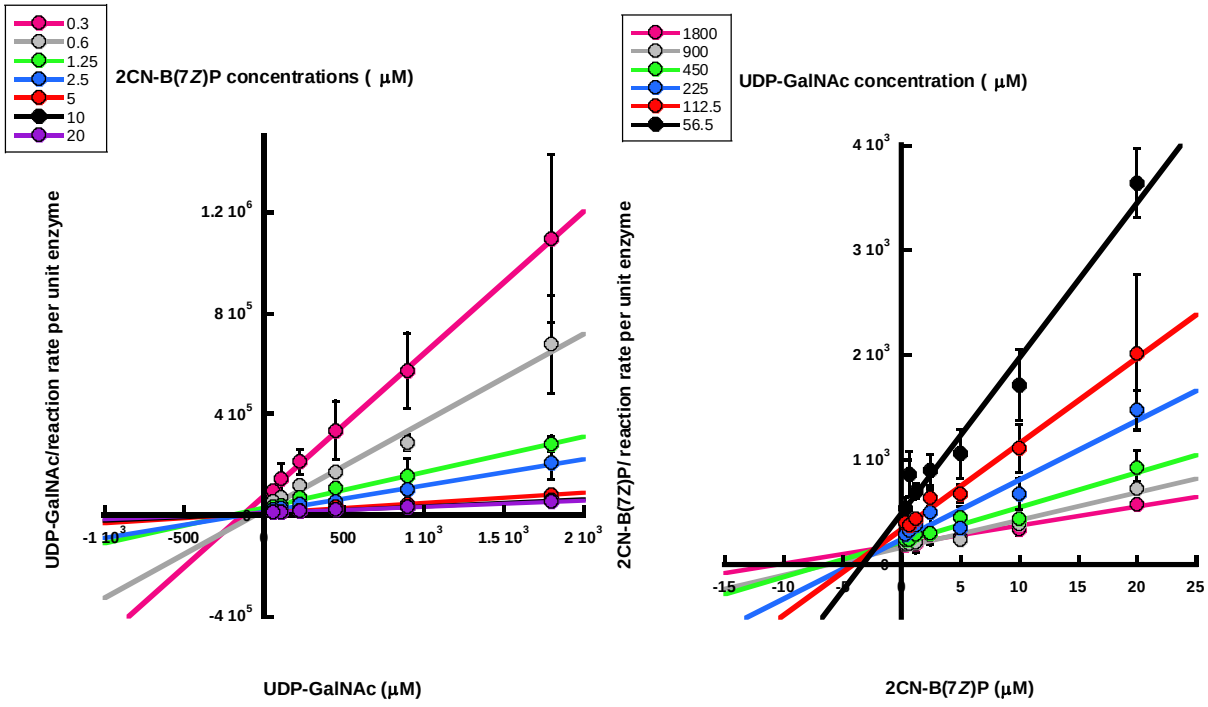


Figure S8. Optimization of surfactant choice on WecP activity. When not varied, standard conditions included the following: 10 mM $MgCl_2$, 10 mM KCl, 200 mM Bicine (pH 8.0), 4 uM 2CN-B(*7Z*)P with 2% n-propanol, 216 uM UDP-GlcNAc, 20 mM DTT, 2.08 μg/mL WecP, and 0.0192% (2.4 xCMC) DDM. Reactions were quenched and analyzed as in Figure S7.



Constant component	Concentration of constant component (µM)	R^2
UDP-GalNAc	56.5	0.9698
	112.5	0.9908
	225	0.9254
	450	0.9463
	900	0.9535
	1800	0.9470
Isoprenoid	0.3	0.9995
	0.6	0.9872
	1.25	0.9935
	2.5	0.9941
	5	0.9768
	10	0.9980
	20	0.9941

Figure S9. Hanes-Woolf plot of WecP kinetic analyses of 2CN-7Z-BP holding isoprenoid constant (left) or UDP-GlaNAc constant (right) with varying concentrations of the other substrate. Reactions were prepared and sampled as described in materials and methods. Fit statistics for each plot is provided in the table.

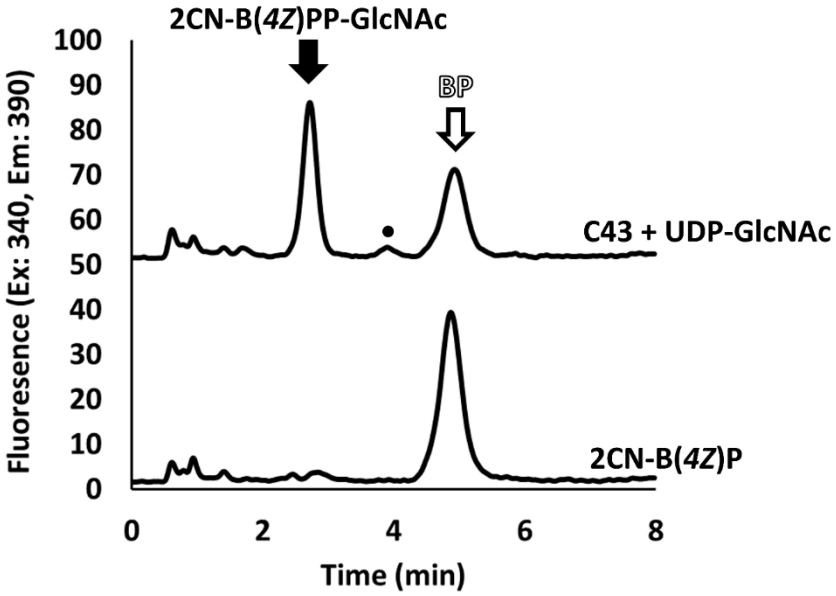


Figure S10. Endogenous WecA activity in cef. The native *E. coli* protein WecA catalyzes the formation of fl-BPP-GlcNAc in the presence of 1 mM UDP-GlcNAc, independent of overexpression.

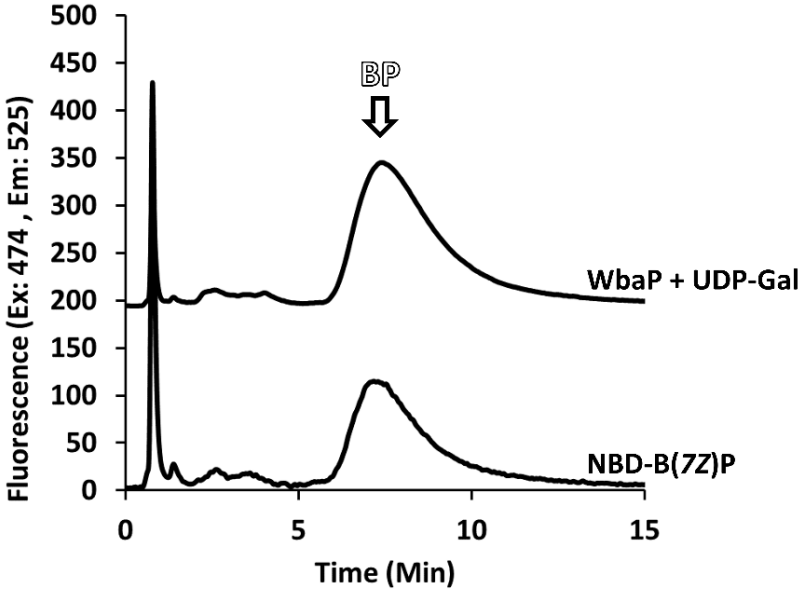


Figure S11. WbaP inactivity with NBD-B(*7Z*)P. Longer polyisopreniods of NBD do not result in transferase activity with WbaP in the presence of cholate.

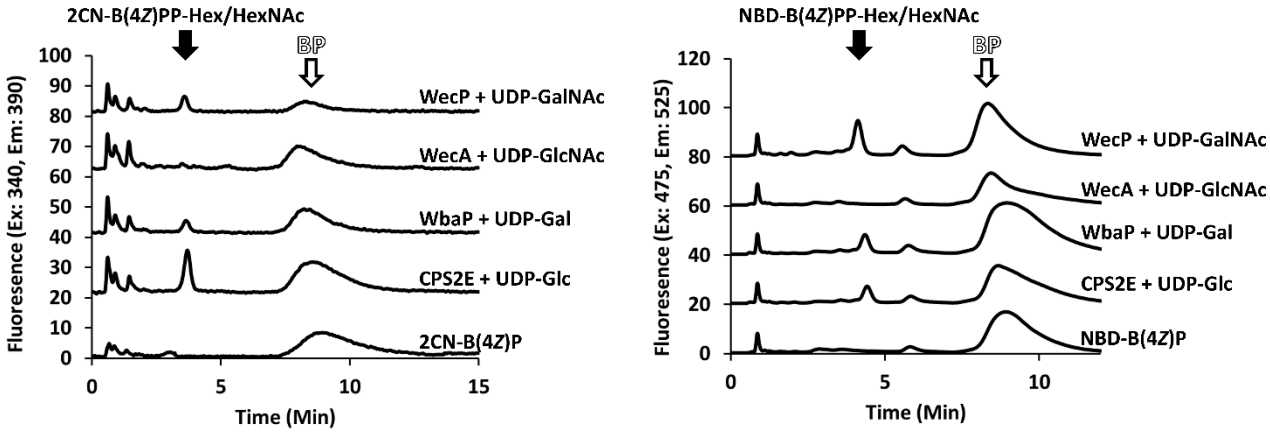


Figure S12. PGT inactivity with *n*-propanol addition. PGT reactions were supplemented with 5% *n*-propanol in the absence of cholate. PGT activity is poor in all cases when propanol is not added with fl-BPs.

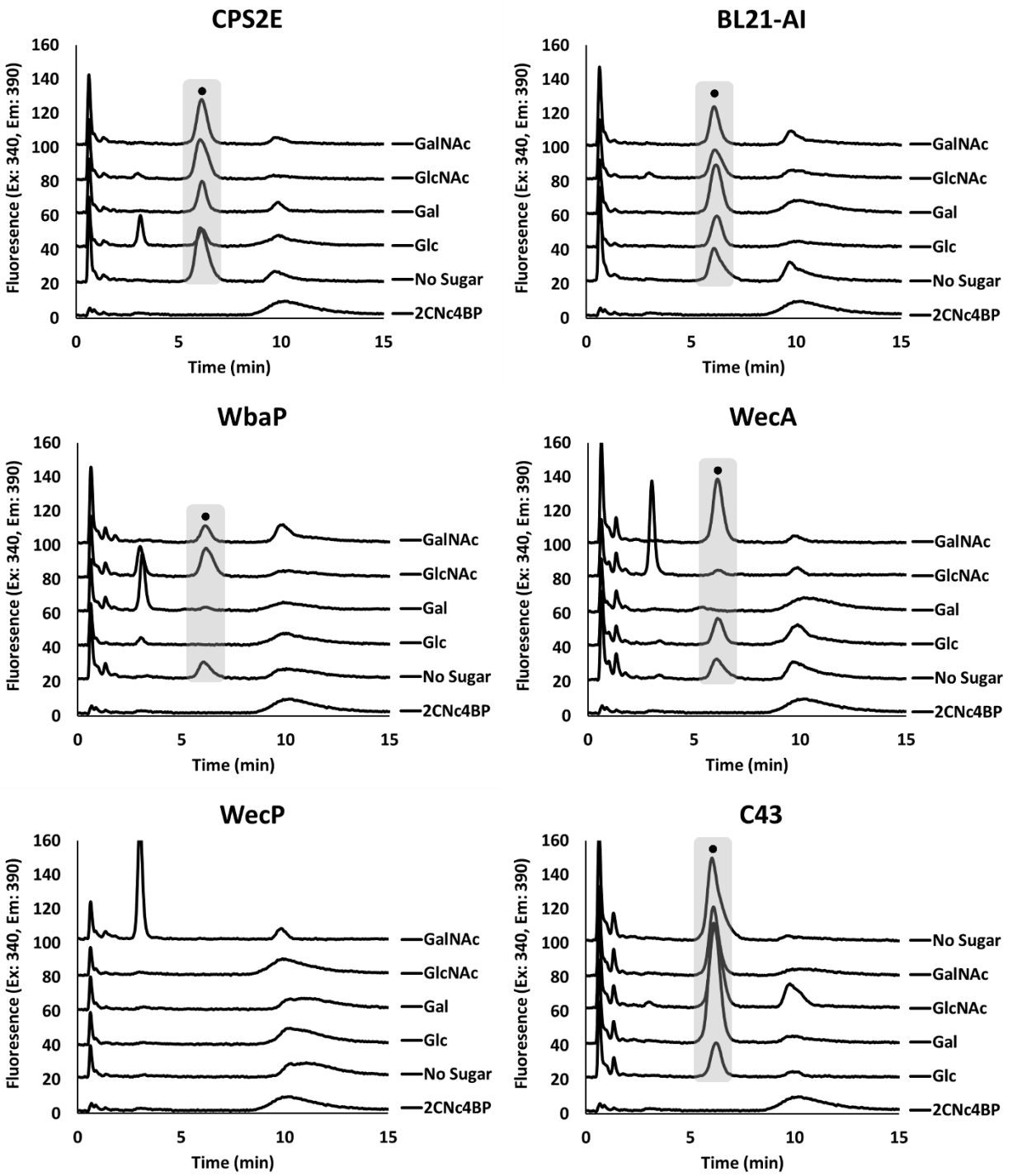


Figure S13. Full glycan donor substrates specificity analysis with 2CN. An additional fluorescence peak (•) is observed at 6.2 min and appears independent of UDP-sugar addition. Injection quantities of each reaction were 40 pmol for 2CN. A co-eluting contaminant is present in the 2CN-B(*4Z*)P standard, which appears at 10 min only after utilization by these enzymes.

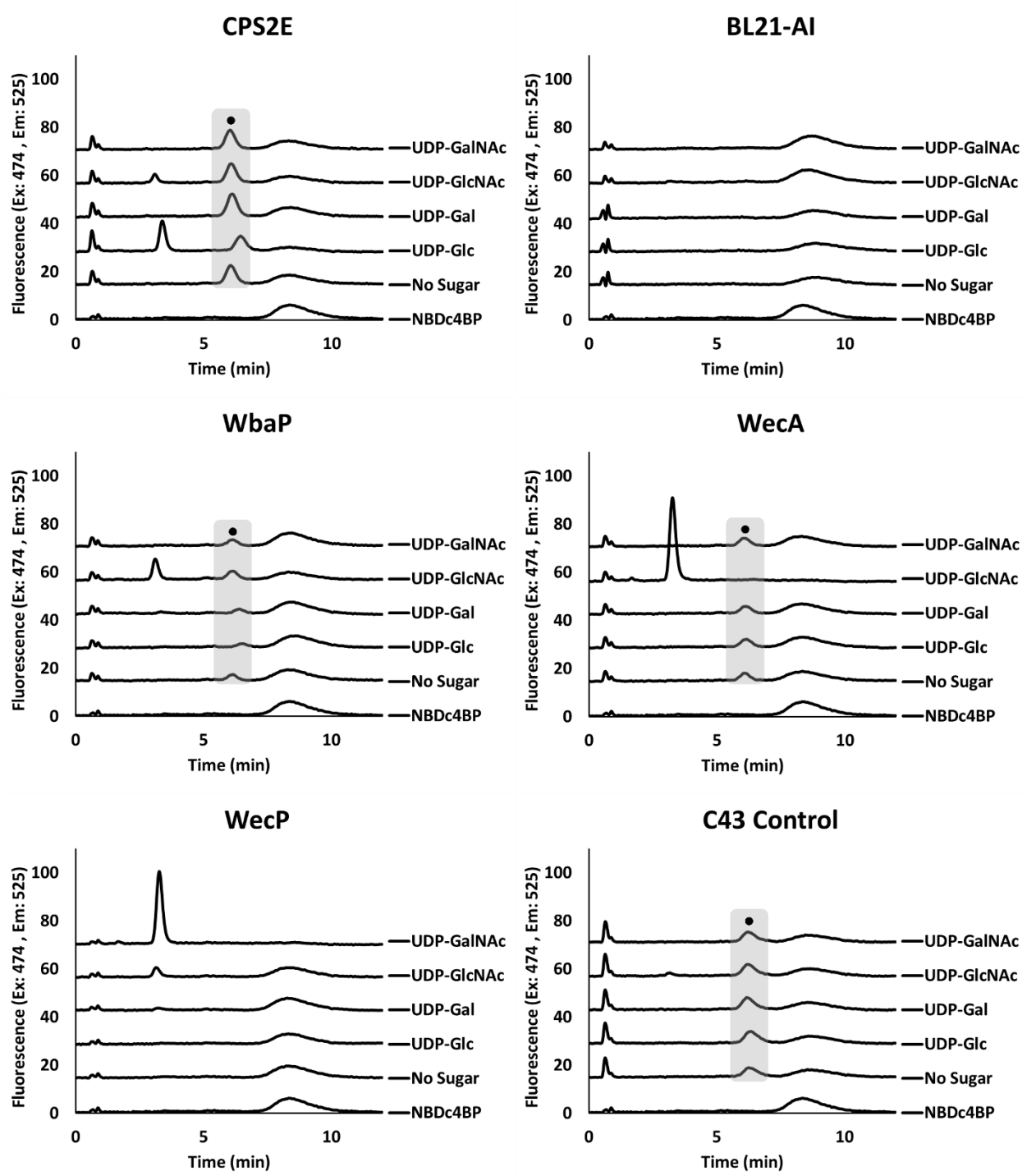


Figure S14. Full glycan donor substrates specificity analysis with NBD An additional fluorescence peak (•) is observed at 6.4 min and appears independent of UDP-sugar addition. Injection quantities of each reaction were 10 pmol.

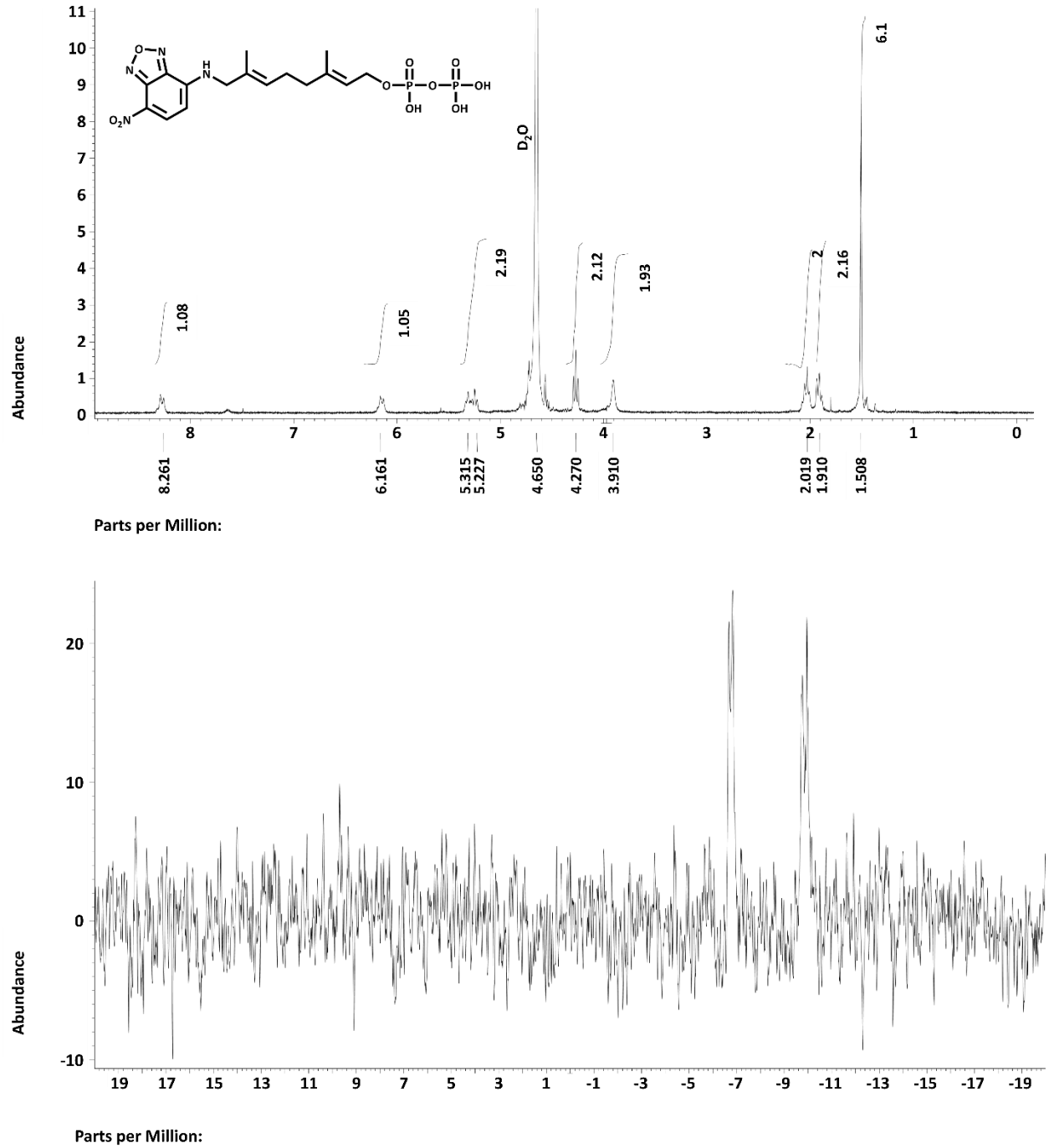


Figure S15. NBD-GPP ^{1}H and ^{31}P NMR tris-ammonium(3,7,-dimethyl-8-(7-nitro-benzo[1,2,5]oxadiazo-4-ylamino)-octa-2,6-dien-1) pyrophosphate (NBD-GPP)

Table S2. Primer sequences used for ΔwecA mutants.

WecA pRED Forward	TGCACAGGACTGGTGGGTTTGGAACGGACTTTCCCTTCTGAATAAAGGTCAATTAACCCTCACTAAAGGGCGG
WecA pRED Reverse	TTATTTGGTTAAATTGGGGCTGCCACCACGATTTCTACGCAGTCTGCGTTTAATACGACTCACTATAGGGCTCG
WecA_ck_Rev	CCCATGCCAATAATCCATAGC

SI Methods

Expression and Purification of UppS. A 5 ml starter culture was prepared overnight. Terrific broth, 1 L, (yeast extract 24 g/L, tryptone 20 g/L, glycerol 4 mL/L, and 90 mM phosphate buffer pH 7.4) supplemented with 1% glucose was inoculated with 1 mL of the overnight culture with 50 μg/mL kanamycin. The culture was then incubated at 37 °C with shaking until an OD_{600} of 0.6 was reached. Overexpression was induced with the addition of 1 mM of IPTG to each culture for 4-6 hours at 30 °C. Cultures were then centrifuged at 10,000 g for 5 min, and cell pellets were suspended in 40 mL of lysis buffer containing 50 mM Tris-HCl pH 8.0, 200 mM NaCl, and 20 mM imidazole. Cells were then lysed via sonication at 25% power for 3 min with a pulse of 1 s on and 2 s off. The resulting lysate was centrifuged under vacuum at 91,635 RCF for 60 min at 4 °C. The supernatant was then added to a column containing 2.0 mL of packed nickel-nitrolotriacetic acid resin (Ni-NTA, PerfectoPro, 5 Prime Inc.) and the lysate was passed through twice. The column was then washed with 20 mL of wash buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, and 50 mM imidazole). The protein was then eluted in six half-column volume fractions (1 mL) with elution buffer (100 mM Bicine pH 8.0, 200 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, and 500 mM imidazole). SDS-PAGE analysis was performed on all fractions (lysate, flow through, wash, and elutions) to determine which fractions contained protein. Elutions containing protein were pooled together and dialyzed thrice in 1 L dialysis buffer (100 mM Bicine pH 8.0, 200 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$) at 4 °C with at least 4 h intervals in between buffer changes. The final concentrations, typically in excess of 500 uM, were calculated spectrophotometrically with an extinction coefficient of ε_{UppSSa} = 36,900 $M^{-1}cm^{-1}$.

Expression, purification, and solubilization of WecP. Bacterial cultures baring p*wecP* were prepared as described above and incubated at 37 °C with shaking until an OD_{600} of 1.0 was reached. Overexpression was induced with the addition of 1 mM of IPTG to each culture for 3 hours, maintaining the temperature at 37 °C. Cultures were then centrifuged at 10,000 g for 5 min, and cell pellets were suspended in 20 mL of buffer containing 50 mM Tris-HCl pH 8.0 and 200 mM NaCl. Cells were then lysed via sonication at 25% power for 3 min with a pulse of 1 s on and 2 s off. The resulting lysate was then briefly centrifuged at 2,500 g for 10 min to remove unlysed cell debris. The supernatant, containing soluble and membrane proteins, was further centrifuged under vacuum at 91,635 RCF for 60 min at 4 °C. The cell envelope fraction was homogenized in 20 mL lysis buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 20 mM imidazole, and 1% Triton-X100) supplemented with 1% Triton-X100. The suspension was then agitated for 4 h at 4 °C. Then, 0.5 mL of nickel-nitrolotriacetic acid resin (Ni-NTA, PerfectoPro, 5 Prime Inc.) was added and the homogenate was agitated 4 h to overnight while at 4 °C. The flow-through was eluted and the column was washed with 12 mL of wash buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 50 mM imidazole, and 1% Triton-X100). Solubilized WecP was eluted from the column in six half-column volume fractions (250 μL) with elution buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 500 mM imidazole, and 1% Triton-X100). SDS-PAGE analysis was performed on all fractions (lysate, flow through, wash, and elution's) to determine which fractions contained protein. Elutions that contained protein were pooled together and dialyzed thrice in dialysis buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl) at 4 °C with at least 4 h intervals between buffer changes. A Bradford assay was used to determine the final total protein content of solubilized WecP, due to possible absorption from Triton-X100.

Expression and cef preparation of CPS2E, WbaP, and WecA. The overexpression of membrane bound proteins was identical to that of WecP, except 250 mL of TB was used. Induction occurred after an OD of 1.0 was reached by the addition of 1 mM IPTG (or 0.2% Arabinose for CPS2E). Cells were induced for 3 h for WbaP and WecA, or 0.5 h for CPS2E (as previously reported). Following induction and culture centrifugation, the cell pellet was suspended in lysis buffer containing 50 mM Tris-HCl pH 8.0, and 200 mM NaCl. Cells were then lysed and centrifuged as described above. The cell envelope fraction was homogenized in 1 mL of the above buffer and was used directly in activity assays. A Bradford assay was used to determine the final total protein content of cef.

ΔWecA C43 Deletion Mutant. *E. coli* MG1655 was transformed with pKD46 and plated on LB + 100 µg/mL ampicillin to select for successful transformants. After a 24 h transformation incubation, 6 colonies from the selection plate were inoculated into 5 mL LB, 100 µg/mL ampicillin, and 0.2% glucose (to repress the arabinose-inducible promoter on pKD46). This culture was grown with shaking at 30 °C overnight, then diluted 1:100 into LB with 100 µg/mL ampicillin and grown shaking at 30 °C. After 2.5 h, 10 mM arabinose was added to induce expression of the *γ*, *β*, and *exo* genes on pKD46. This induction proceeded for 1 hr, then the culture was centrifuged, then the pellet was washed twice in 0.3 M sucrose, and finally resuspended in 0.3 M sucrose at 1/50 the original culture volume. These induced, electrocompetent cells were stored at -80 °C until use.
Primers were designed to amplify the FRT-flanked neo^R gene (conferring kanamycin resistance, from the pRED/ET kit, Gene Bridges) and also append 50 base pairs homologous to the genome sequence flanking the *wecA* gene in *E. coli*. Gel electrophoresis confirmed a PCR amplicon of 1,737 base pairs. Next, 2 µg of this amplicon was combined with 50 µL of the previously prepared electrocompetent MG1655 pKD46 cell preparation and electroporated at 2,500 V. SOC media (1 mL) was added immediately following electroporation and cells were recovered at 37 °C for 2.5 h. This reaction was plated on LB with 50 µg/mL kanamycin, and incubated at 37 °C overnight. The following day, colonies that grew on this selective media were restreaked on LB with 50 µg/mL kanamycin to purify, then PCR was used to confirm the correct insertion using the primers WecA_pRED_Fwd and WecA_ck_Rev (**SI Tabel 2**), which produced a 1,858 base pair product by gel electrophoresis, indicating insertion of the amplicon in place of the native *wecA* gene. This mutation was then moved into a C43 background by P1 transduction, and propagated in LB/Kan to select for Δ*wecA*.

General
All reagents were ACS grade or higher and purchased from Sigma-Aldrich, VWR, or Fisher Scientific unless otherwise stated. TLC was performed on fluorescent plates and *p*-anisaldehyde stain was used for detection. Preparation of NBD-GPP has been adapted from the literature with some modifications.[1-3] Preparation of IPP and tris(tetra n-butyl ammonium) diphosphate (TTNBA-PP) have been previously reported.[4] Characterization of all compounds were carried out on 500 MHz Jeol for ^{1}H and ^{13}C NMR for intermediates, and ^{1}H and ^{31}P for final products (NBD-GPP, IPP, and TTNBA-PP).

Preparation of 2-[[(2E)-3,7-dimethyl-2,6-octadienyl]oxy]tetrahydropyran (HO-G-THP)

To a round bottom flask, 1 equivalent of geraniol, 1.5 equivalents of 3,4-dihydropyrane and 0.1 equivalents of PPTS was added in dichloromethane at room temperature for 12 h. The solvent was then removed and the crude reaction was placed in a separatory funnel and extracted with diethyl ether and brine. The organic layer was collected and dried with sodium sulfate for 1 h resulting in quantitative yield of G-THP. The crude product was analyzed by TLC in hexanes/ethyl acetate (3:1) and visualized with p-anisaldehyde ($R_f = 0.78$).

Preparation of (2E,6E)-2,6-Dimethyl-8-[(tetrahydro-2H-pyran-2-yl)oxy]-2,6-octadien-1-ol (HO-G-THP)

Into a round bottom was added 4 equivalents of tert-butyl hydroperoxide 70% (v/v), 0.1 equivalent of salicylic acid, and 0.1 equivalent of selenium dioxide at room temperature until homogenous. The solution was cooled to 0 °C, after which 1 equivalent of G-THP was added. The reaction was allowed to sit for 36 h after which time the solvent was removed. The resulting oil was then placed into a separatory funnel with ethyl ether and washed with 5% (w/v) sodium bicarbonate, saturated copper sulfate, saturated thiosulfate (X2), water, and then brine. The organic layer was then collected and dried with sodium sulfate for 1 h. The crude reaction was analyzed by TLC in hexanes/ethyl acetate (7:3; $R_f = 0.24$). The crude reaction was purified by flash chromatography over silica gel with from hexanes until remaining G-THP was eluted. Then hexanes/ethyl acetate (4.5:1) was used to elute the aldehyde product. Lastly, the desired alcohol product (HO-G-THP) was eluted with hexanes/ethyl acetate (7:3) with typical yields of 20-30 %.

Preparation of 2-[(2E,6E)-2,6-Dimethyl-8-[(tetrahydro-2H-pyran-2-yl)oxy]-2,6-octadien-1-yl]-1H-isoindole-1,3(2H)-dione (Pt-G-THP)

Into a round bottom flask 1 equivalent of phthalimide, 1 equivalent of triphenylphosphine (recrystallized from hot ethanol) and 1 equivalent of HO-G-THP was added with diethyl ether. The slurry was cooled in a salted ice bath. One equivalent of diethyl azodicarboxylate (DEAD, 40 % in toluene) was added dropwise and the reaction was left to warm to room temperature for 12 h after which time the solvent was removed. The solids were removed by filtration, and the solvent removed. Product formation, Pt-G-THP, was monitored by TLC in hexanes/ethyl acetate (3:1; $R_f = 0.46$, 60% yield) and purified with hexane/ethyl acetate (6:1).

Preparation of (2E,6E)-2,6-Dimethyl-8-[(tetrahydro-2H-pyran-2-yl)oxy]-2,6-octadien-1-amine (NH_2-G-THP)

Into a round bottom flask, 1 equivalent of Pt-G-THP, 6 equivalents of hydrazine monohydrate and dry ethanol (0.04 mmol mL^{-1}) was added and left at room temperature for 12 h. The solution was filtered and the solvent removed after this time. The reaction was purified and analyzed by TLC with a mixture of chloroform:methanol:ammonium hydroxide (100:10:1, 42% yield). TLC plates were pre-treated in this mixture by allowing them to saturate in the TLC chamber and then dry completely prior to use.

Preparation of 7-nitrobenzo(1,2,5)oxadiazol-4-yl-1-(2,6-dimethyl-8-(tetrahydro-pyrane-2-yloxy)- 2,6-octadien-1-amine (NBD-G-THP)

To a round bottom flask containing acetonitrile/25 mM sodium bicarbonate buffer (1:1), 1 equivalent of H_2N-G-THP was added. A solution of 1.5 equivalents of NBD-Cl in acetonitrile was added dropwise. The mixture was stirred for 1 h at room temperature and a second solution

of 1.5 equivalents of NBD-Cl in acetonitrile was added dropwise for 1 h. The solution was then poured into a separatory funnel and extracted with methylene chloride and washed with brine solution. The organic layer was collected and then dried with sodium sulfate for 1 h and then the solvent was removed. The reaction was monitored and purified with methylene chloride with theoretical yield (R_f = 0.18, 84% yield).

Preparation of 3,7,-dimethyl-8-(7-nitro-benzo[1,2,5]oxadiazo-4-ylamino)-octa-2,6-dien-1-ol (NBD-GOH)

To a round bottom flask, 1 equivalent of NBD-G-THP was dissolved in ethanol. The reaction was heated to 60 °C for 3 h. The reaction was transferred to a separatory funnel and extracted with diethyl ether and washed with brine solution. The organic layer was collected and dried with sodium sulfate and filtered. The solvent was removed and purified with cyclohexane/ethyl acetate (1.5:1) and the reaction was monitored with hexanes/ethyl acetate (1.5:1; R_f = 0.1, 98-100% yield).

Preparation of tris-ammonium(3,7,-dimethyl-8-(7-nitro-benzo[1,2,5]oxadiazo-4-ylamino)-octa-2,6-dien-1) pyrophosphate (NBD-GPP)

To a flame dried flask under argon, 1 equivalent of NBD-G-OH and 0.5 equivalents of 1 M PBr3 in dichloromethane was added. The reaction was immediately checked by TLC in hexanes/ethyl acetate (7:3) for the formation of the bromide. Three equivalents of tris(tetra-butylammonium) diphosphate in acetonitrile was added upon formation of the bromide. The reaction was allowed to sit for no more than one hour and was monitored by TLC (R_f = 0). The volatiles were then removed without the use of heat. The crude product was then added to an ion exchange column (ammonium form), followed by two 0.5 mL washes of 25 mM ammonium bicarbonate. The column was eluted with ammonium bicarbonate buffer and the flow through was monitored for a drop in pH to pH 4. The elutant was collected until pH 7 and lyophilized overnight. The solid was dissolved in 1 mL of ammonium bicarbonate with acetonitrile (80:20) and purified by reverse phase HPLC as previously reported (15-24 % yield). ^{1}H NMR (300 MHz) in D_20: δ 8.26 (d, 1H), 6.16 (d, 1H), 5.31 (t, 1H), 5.23 (t, 1H), 4.27 (t, 2H), 3.91 (s, 2H), 2.03 (t, 2H), 1.91 (t, 2H), 1.51 (s, 6H). ^{31}P NMR (300 MHz): -7.2 (d, 1P), -9.8 (d, 1P).

References

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