Supporting Information, "Plant EPSP synthase desensitized to glyphosate: Optimized global sequence context accommodates a glycine to alanine change in the active site"
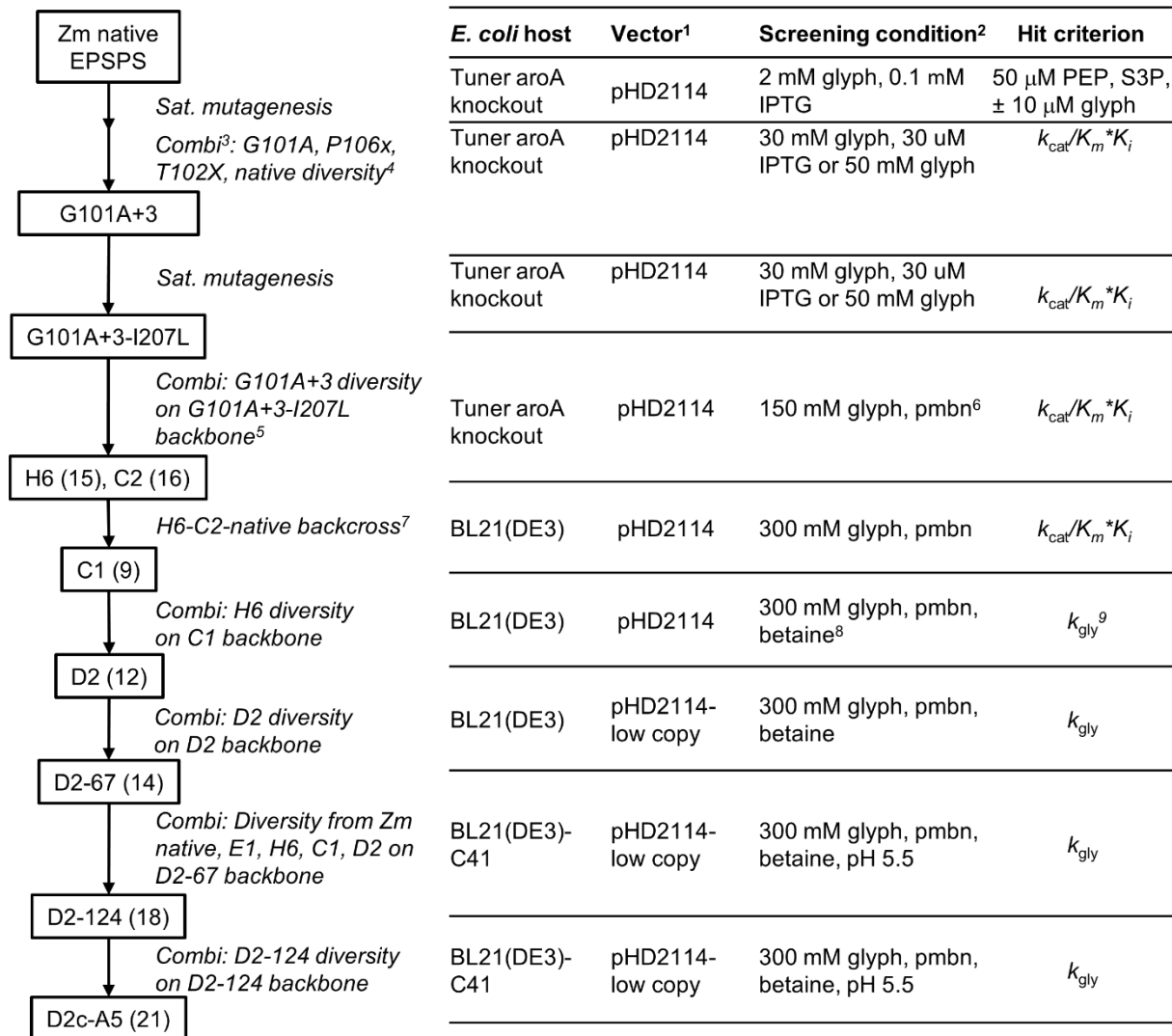
Yuxia Dong, Emily Ng, Jian Lu, Tamara Fenwick, Yumin Tao, Sean Bertain, Marian Sandoval, Ericka Bermudez, Zhenglin Hou, Phil Patten, Michael Lassner, Daniel Siehl

Contents
1. Flow path for optimizing maize EPSPS (Figure S1)
2. Slow release of glyphosate from native maize EPSPS (Figures S2-S5)
3. Map of *E. coli* expression vector

1. Flow path for optimizing maize EPSPS
Figure S1. Flow path for optimizing maize EPSPS

Boxes and optimization process (flow path, top to bottom):

- Zm native EPSPS
  - *Sat. mutagenesis*
  - *Combi[3]: G101A, P106x, T102X, native diversity[4]*
- G101A+3
  - *Sat. mutagenesis*
- G101A+3-I207L
  - *Combi: G101A+3 diversity on G101A+3-I207L backbone[5]*
- H6 (15), C2 (16)
  - *H6-C2-native backcross[7]*
- C1 (9)
  - *Combi: H6 diversity on C1 backbone*
- D2 (12)
  - *Combi: D2 diversity on D2 backbone*
- D2-67 (14)
  - *Combi: Diversity from Zm native, E1, H6, C1, D2 on D2-67 backbone*
- D2-124 (18)
  - *Combi: D2-124 diversity on D2-124 backbone*
- D2c-A5 (21)

E. coli host	Vector[1]	Screening condition[2]	Hit criterion
Tuner aroA knockout	pHD2114	2 mM glyph, 0.1 mM IPTG	50 μM PEP, S3P, ± 10 μM glyph
Tuner aroA knockout	pHD2114	30 mM glyph, 30 uM IPTG or 50 mM glyph	k_{cat}/K_m*K_i
Tuner aroA knockout	pHD2114	30 mM glyph, 30 uM IPTG or 50 mM glyph	k_{cat}/K_m*K_i
Tuner aroA knockout	pHD2114	150 mM glyph, pmbn[6]	k_{cat}/K_m*K_i
BL21(DE3)	pHD2114	300 mM glyph, pmbn	k_{cat}/K_m*K_i
BL21(DE3)	pHD2114	300 mM glyph, pmbn, betaine[8]	k_{gly}[9]
BL21(DE3)	pHD2114-low copy	300 mM glyph, pmbn, betaine	k_{gly}
BL21(DE3)-C41	pHD2114-low copy	300 mM glyph, pmbn, betaine, pH 5.5	k_{gly}
BL21(DE3)-C41	pHD2114-low copy	300 mM glyph, pmbn, betaine, pH 5.5	k_{gly}

Boxes indicate an EPSPS variant with number of mutations in parentheses
Arrows with descriptions in italics indicate an optimization process (saturation mutagenesis or combinatorial library).
The table specifies the screening procedure for the adjacent library.

[1]The vector used for expression in *E. coli*, described in Methods; "low copy" indicates that the *ori* is
   exchanged with that of pSC101, generating ~5 copies per cell rather than ~20.
[2]Amendment added to the minimal basal medium described in Methods
[3]Combi: Combinatorial library of the diversity indicated
[4]Diversity: The neutral or beneficial substitutions identified by saturation mutagenesis
[5]Backbone: The amino acid sequence upon which the combinatorial library is built
[6]pmbn: Polymyxin B-sulfate nonapeptide, supplied at 1 mg/L
[7]H6-C2-native backcross: See Supporting Information, next section, for details
[8]betaine: Supplied at 1 mM
[9]k_{gly} is enzyme turnover, min^{-1}, under simulated *in vivo* application conditions (30 μM PEP, 30 μM S3P
and 1 mM glyphosate; for rationale, see Results).

2. Slow release of glyphosate from native maize EPSPS

The usual method we used to perform substrate saturation kinetic analysis was to place the varied
substrate into the wells of the assay plate and start reactions by the addition of a mixture of all other
components (enzyme, buffer, fixed substrate, coupling reagents and if appropriate, glyphosate). With that
procedure for saturation with PEP, we obtained data that best fit non-competitive inhibition (Figure S2).

Figure S2. Non-competitive inhibition when native maize EPSPS is pre-mixed with S3P and glyphosate
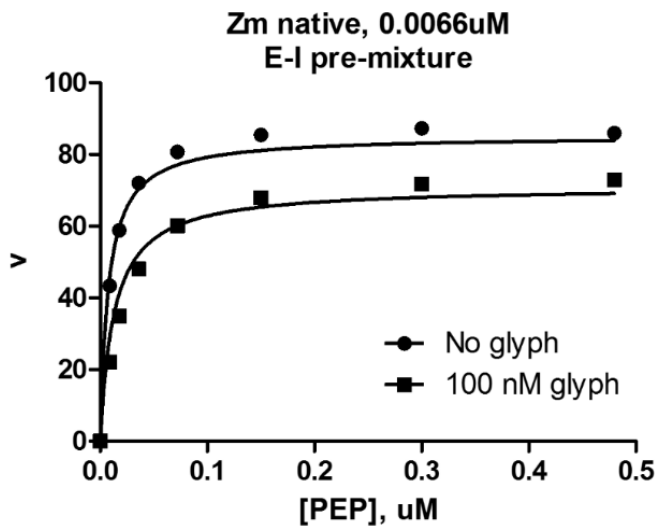
The explanation for that anomalous result was the observation of slow recovery of activity from an
E:S3P:glyphosate complex upon addition of PEP, suggesting slow dissociation of glyphosate from the
complex (Figure S3, left panel).

Figure S3. Slow release of glyphosate from a previously formed E:S3P:glyph complex
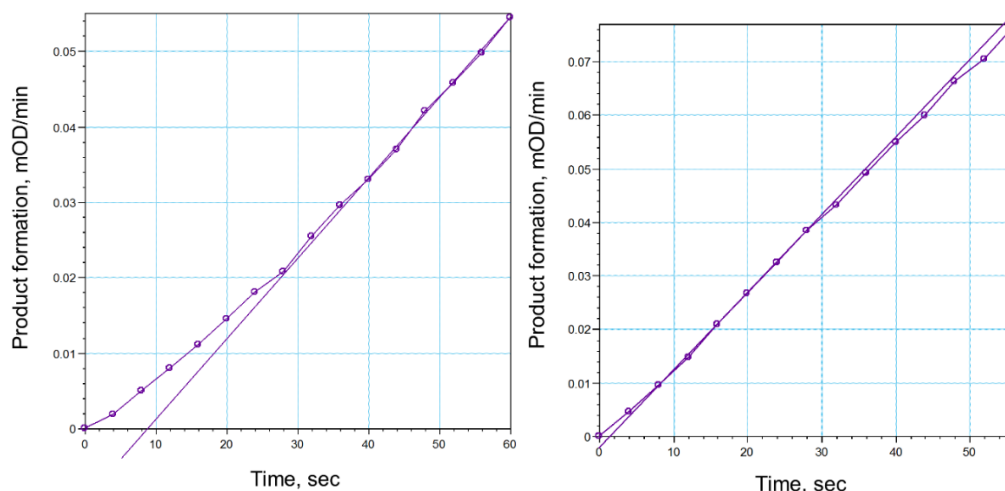
Left panel; enzyme was mixed with 100 nM glyph and 0.14 mM S3P before starting the reaction with the addition of 0.3 mM PEP.
Right panel; same reaction conditions except that glyphosate was omitted.

The same plot theoretically could be due to insufficient catalytic capacity of the coupling enzyme, purine nucleotide phosphorylase. That this was not the case is shown in Figure S3, right panel, where the same reaction conditions without glyphosate, resulted in a linear time course.
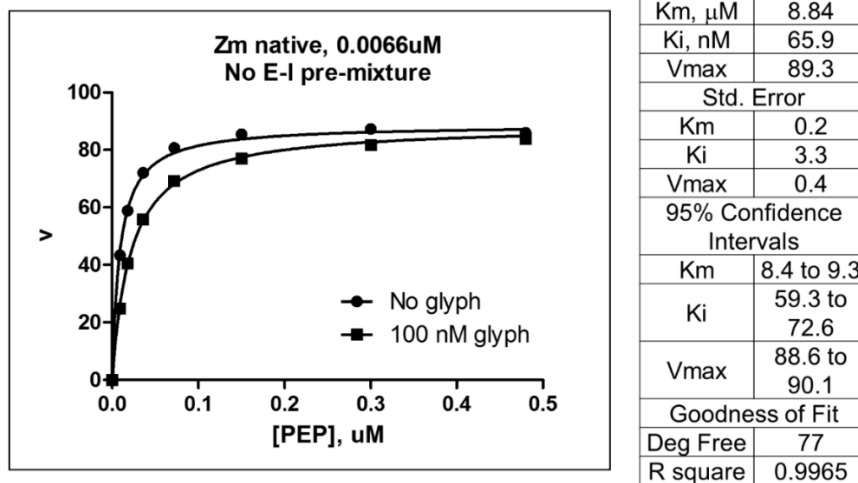
Under the hypothesis that the non-competitive plot was due to a fraction of the enzyme being bound with glyphosate during rate measurement, the procedure was altered such that the enzyme was exposed to PEP and glyphosate simultaneously. This was done by placing PEP and glyphosate (5 μl each) in the wells of the assay plate, then starting the reaction with the addition of the mixture of enzyme, S3P and the other reaction components. With that modification, an accelerating reaction rate, inferring slow release of glyphosate from an E:S3P:glyph complex, was not seen (Figure S4).

Figure S4. Reaction time course with native maize EPSPS and S3P added to a well containing PEP and glyphosate. Final concentrations of PEP, S3P and glyphosate were 0.3 mM, 0.14 mM and 100 nM, respectively.
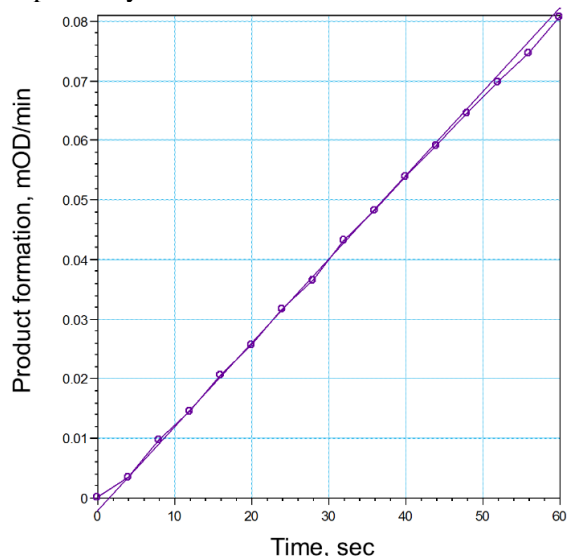
Global non-linear regression analysis of a family of such reactions with varying concentrations of PEP, with or without 100 nM glyphosate resulted in an excellent fit with the expected equation for competitive inhibition (Figure S5). None of the other variants in this study exhibited slow dissociation of glyphosate from an E:S3P:glyphosate complex.

Figure S5. Competitive inhibition when maize native EPSPS is exposed simultaneously to PEP and glyphosate. Saturation kinetics with varying PEP, 100 nM glyphosate.

Km, μM	8.84
Ki, nM	65.9
Vmax	89.3
Std. Error	
Km	0.2
Ki	3.3
Vmax	0.4
95% Confidence Intervals	
Km	8.4 to 9.3
Ki	59.3 to 72.6
Vmax	88.6 to 90.1
Goodness of Fit	
Deg Free	77
R square	0.9965

3. Map of *E. coli* expression vector

Figure S6. pHD2114, the vector used for expressing EPSPS variants in E. coli. The cloning site for all variants is here shown occupied by the nucleotide sequence coding for native maize EPSPS.