

## *Supplementary Material*

### **Immobilization of the highly active UDP-glucose pyrophosphorylase from *Thermocrispum agreste* provides a highly efficient biocatalyst for the production of UDP-glucose**

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The supplemental material comprises the following information; codon optimized gene encoding the employed biocatalyst as well as activity data regarding divalent cations, buffers, potential inhibitors, solvents and kinetic data describing soluble and immobilized enzyme preparations.

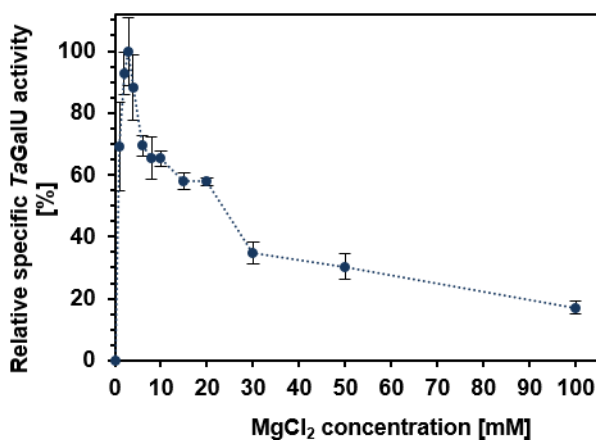
The *TaGalU* gene (accession number: WP\_028847555) was codon optimized (accession number: MT321102) and synthesized prior cloning.

>codon optimized gene plus restrictions sites for cloning (*NdeI* and *NotI*; underlined)

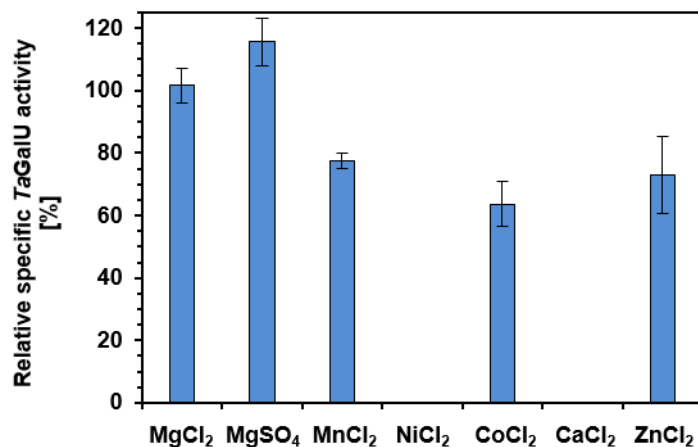
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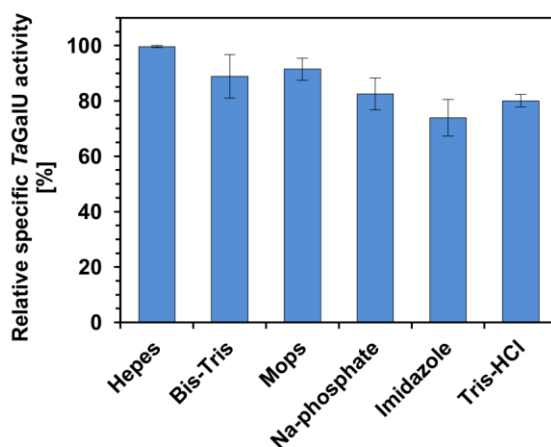
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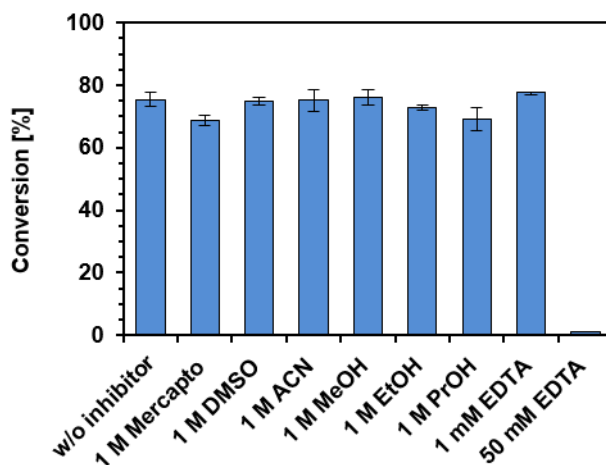
**Supplementary Figure 1. Relative specific *TaGalU* activity depending on the applied magnesium chloride concentration.** Reaction solution contained 2 mM UTP, 2 mM G1P, 0-100 mM MgCl<sub>2</sub>, 50 mM Hepes, pH 7.0, 0.013 µg mL<sup>-1</sup>, 50 °C, 1 mL reaction volume. 100 % relative activity corresponds to 2111 U mg<sup>-1</sup>. Means with standard deviations of triplicate measurements are shown.



**Supplementary Figure 2. Different metals as cofactors tested in the *TaGalU* reaction.** 1 mL reaction solution contained 2 mM UTP, 30 mM G1P, 3 mM metal salt, 50 mM Hepes, pH 7.0 and 0.013  $\mu\text{g mL}^{-1}$  *TaGalU*. Reaction was carried out at 50 °C. 100 % corresponds to 1256 U  $\text{mg}^{-1}$ . Means with standard deviations of triplicate measurements are shown.



**Supplementary Figure 3. Different buffers tested for the *TaGalU* reaction.** 1 mL of reaction mixture contains 2 mM UTP, 30 mM G1P, 3 mM MgCl<sub>2</sub>, 50 mM buffer, pH 7.0 and 0.013  $\mu\text{g mL}^{-1}$  *TaGalU*. The reaction temperature was 50 °C. 100 % corresponds to 1258 U  $\text{mg}^{-1}$ . Means with standard deviations of triplicate measurements are shown.



**Supplementary Figure 4. Effect of inhibitors or solvents to *TaGalU*.** The reaction mixture contained: 2 mM UTP, 30 mM G1P, 3 mM  $\text{MgCl}_2$ , 50 mM Hepes, pH 7.0, 1 mM and 50 mM EDTA, respectively or 1 M solvent/inhibitor,  $0.013 \mu\text{g mL}^{-1}$  *TaGalU*. Reaction was carried out at 50 °C in 200  $\mu\text{L}$  reaction volume. Means with standard deviations are shown. Mercapto: 2-mercaptoethanol, DMSO: dimethyl sulfoxide, ACN: acetonitrile, MeOH: methanol, EtOH: ethanol, PrOH: isopropanol, EDTA: ethylenediaminetetraacetic acid.

**Supplementary Table 1. Kinetic constants of the free and immobilized *TaGalU* calculated by fits**

kinetic constant	Free		Immobilized		Immobilized and multiplied by factor	
	UTP	G1P	UTP	G1P	UTP	G1P
$K_m$ [mM]	0.15	0.12	0.40	0.79	0.40	0.79
$V_{\max}$ [U $\text{mg}^{-1}$ ]	1698	1109	226	157	3480 <sup>a</sup>	2418 <sup>a</sup>
$k_{\text{cat}}$ [ $\text{s}^{-1}$ ]	914	597	121	84	1874 <sup>a</sup>	1302 <sup>a</sup>
$K_I$ [mM]	3.9	-	3.9	-	3.9	-
$k_{\text{cat}}/K_m$ [ $\mu\text{M}^{-1} \text{s}^{-1}$ ]	6.09	4.98	0.3	0.11	4.69 <sup>a</sup>	1.65 <sup>a</sup>

<sup>a</sup>: For data collection of the kinetics for the immobilized *TaGalU* an old batch of enzyme with reduced specific activity was used. Therefore, we recalculated the values for  $V_{\max}$  with the time factor 15.4. This factor emerged from the fact that the initial specific activity of the free enzyme was  $1016 \text{ U mg}^{-1}$  directly after purification. After storage of the enzyme for 1.5 years the activity decreased to only  $66.03 \text{ U mg}^{-1}$ , which is a reduction by the factor 15.4. Therefore, these values are of theoretical nature.