

## Enzymatic Cascade Approaches for Short-Chain Cellodextrin Biosynthesis

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### 1 Materials and Methods

#### Gene selection and cloning

Sucrose phosphorylase (ScP) from *Bifidobacterium adolescentis* (BaScP, GenBank identifier AF543301.1) used here was previously reported and characterized [1, 2]. The gene sequence was codon-optimized for its expression in *Escherichia coli* (subject sequence in Supporting Figure S1). The synthetic gene was ordered from GenScript (Piscataway, New Jersey, U.S.), sub-cloned into pQE30 from Qiagen (Hilden, Germany, Supporting Figure S1). The recombinant enzyme, carrying an N-terminal His-tag (MRGSHHHHHHGS-), has a calculated molecular mass of 56.5 kDa (Supporting Figure S2). The enzyme was characterized for efficient recombinant protein production and glucosyl transfer activity from sucrose to  $\alpha$ -D-glucose 1-phosphate ( $\alpha$ Glc1-P) at pH 7.0. Further biochemical and kinetic characterization studies of BaScP are described in the manuscript.

#### Thermodynamic analysis

The Gibbs free energy change ( $\Delta G$ ) in each step of the cascade reaction was calculated based on the Eq. 1, where  $R$  is the gas constant (8.314 J/(mol·K)) and temperature ( $T$ ) was used at 298.15 K (25 °C),  $K_{eq}$  is the corresponding equilibrium constant of each phosphorylase reaction.

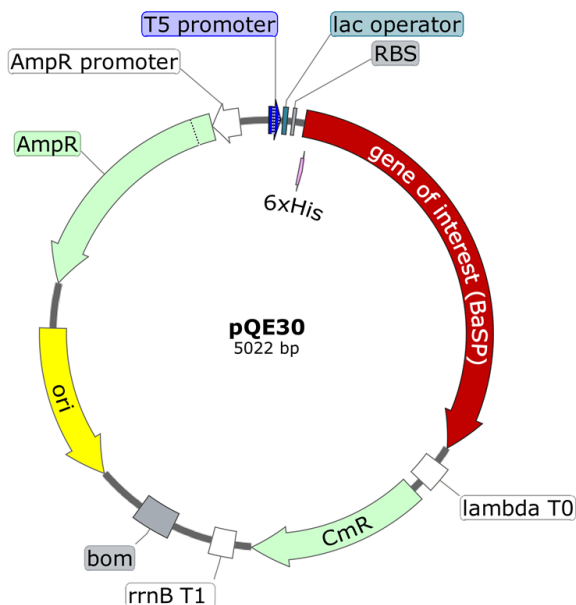
$$\Delta G = -RT \ln K_{eq} \quad (1)$$

For sucrose phosphorylase reaction (sucrose + phosphate  $\leftrightarrow$   $\alpha$ Glc1-P + fructose), the  $K_{eq}$  was obtained from literature [3], where a  $K_{eq}$  of 9.0 was measured (pH 7.0). The  $\Delta G$  for this reaction was thus calculated as -5.4 kJ/mol. Similarly, the cellobiose phosphorylase reaction ( $\alpha$ Glc1-P + glucose  $\leftrightarrow$  cellobiose + phosphate) exhibited a  $\Delta G$  of -3.7 kJ/mol (pH 7.0) calculated from the reported  $K_{eq}$  of 4.3 [4].

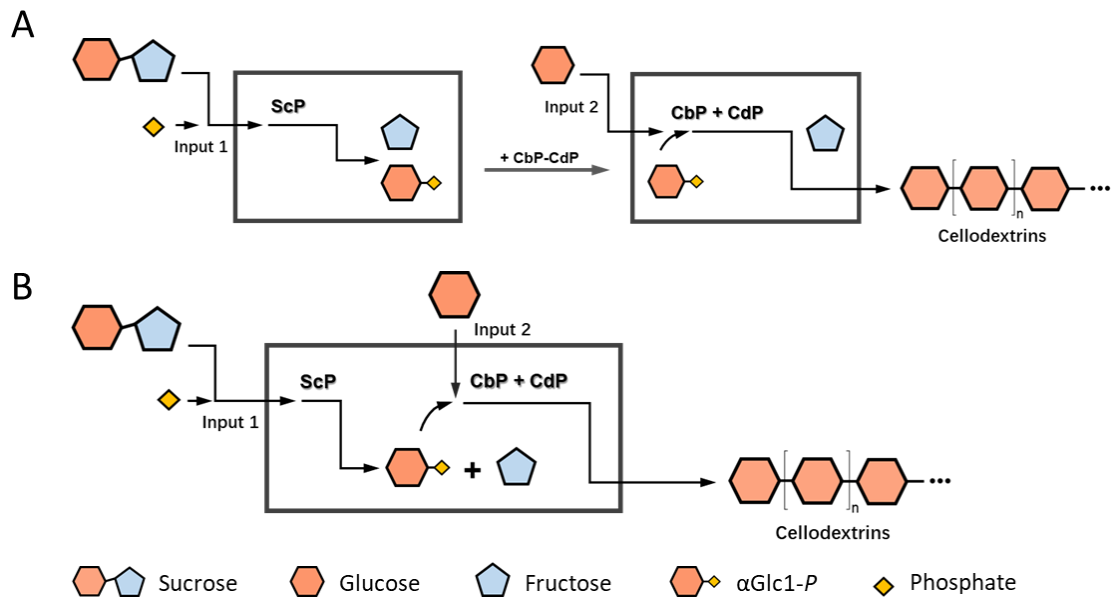
For cellodextrin phosphorylase, the  $K_{eq}$  was determined from a series of experiments that used different initial concentrations of cellobiose (4-20 M) and  $\alpha$ Glc1-P (1-5 mM) dissolved in 50 mM MES buffer, pH 7.0. The reaction to give cellotriose as the only (or major) cellodextrin product ( $\alpha$ Glc1-P + cellobiose  $\leftrightarrow$  cellotriose + phosphate) at apparent equilibrium was used for the  $K_{eq}$  determination. At equilibrium, the final concentration of phosphate was measured, and  $K_{eq}$  was accordingly calculated. A value of 2.3 was obtained. The reaction thus exhibited a  $\Delta G$  of -2.1 kJ/mol.

## 2 Figures and Schemes

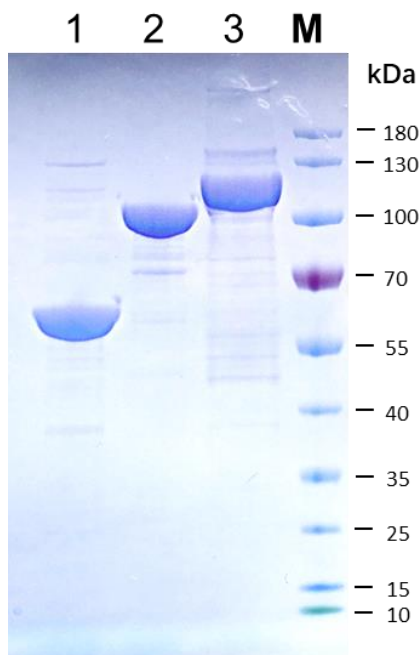
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**TGACCATCGCTCCGACGACCTGATCGCTAATCCGCCGTTGTTGCGTAA**



**Supporting Figure S1.** Codon-optimized gene sequence of the sucrose phosphorylase from *Bifidobacterium adolescentis* (BaScP, GenBank identifier AF543301.1). Start and stop codon are marked in bold (upper panel). The synthetic gene was ordered from GenScript (Piscataway, New Jersey, U.S.) for sub-cloning into pQE30 (lower panel) from Qiagen (Hilden, Germany).

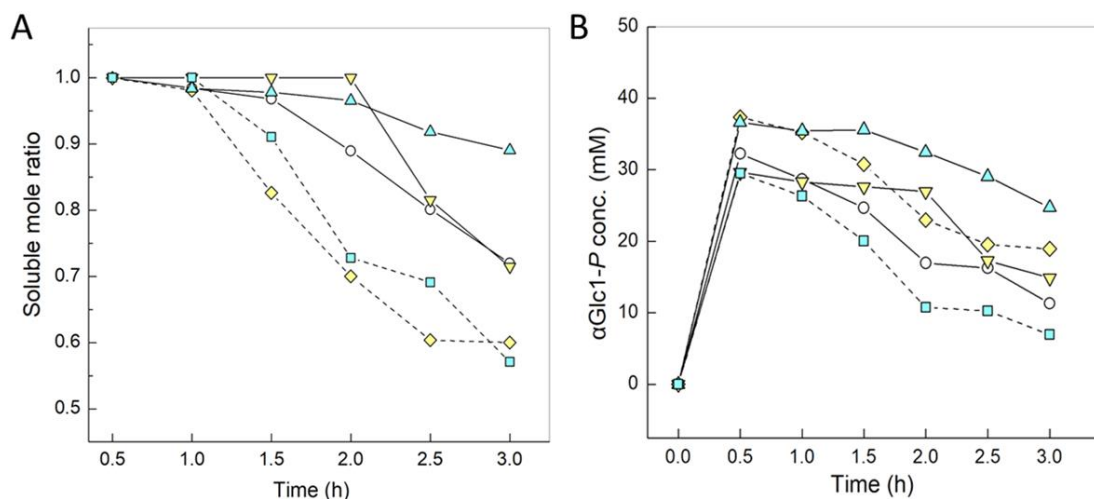


**Supporting Figure S2.** Scheme of the cascade reaction designed for the synthesis of cellodextrins from sucrose and glucose: **A**) sequential two-step reaction; **B**) simultaneous reaction. For the soluble cellodextrins ( $DP \leq 6$ ),  $n$  is 1 - 4. ScP, sucrose phosphorylase; CbP, cellobiose phosphorylase; CdP, cellodextrin phosphorylase.

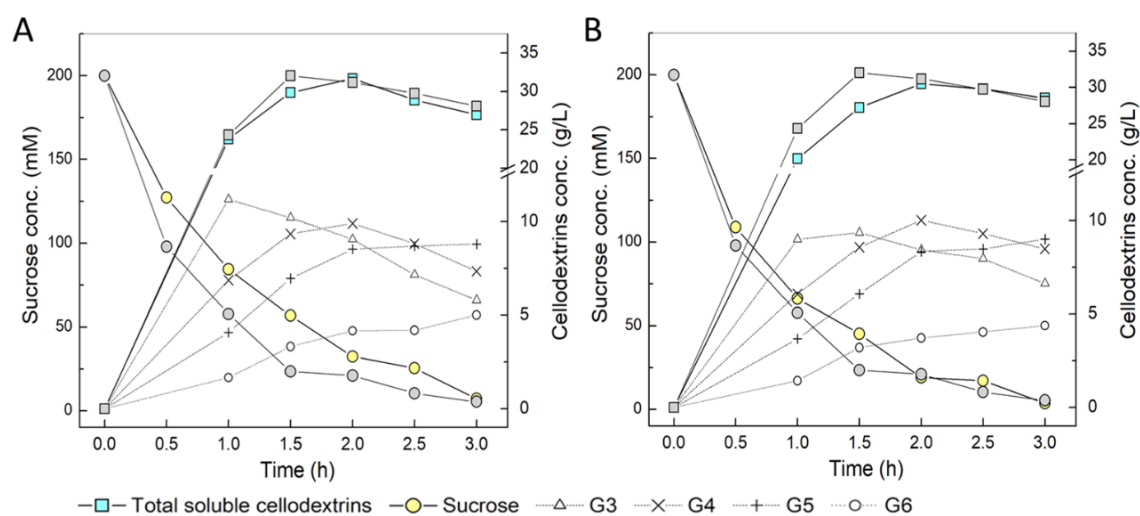


**Supporting Figure S3.** SDS polyacrylamide gel showing the purified recombinant enzymes expressed in *E. coli*. **M**, PageRuler™ Prestained Protein Ladder (10-180 kDa); **1**, purified sucrose phosphorylase from *B. adolescentis* (*BaScP*); **2**, purified cellobiose phosphorylase from *Cellulomonas uda* (*CuCbP*); **3**, purified cellodextrin phosphorylase from *Clostridium cellulosi* (*CcCdP*).

## Metal Ions in Life Sciences



**Supporting Figure S4.** Time-course analysis on **A)** cellodextrins solubility and **B)**  $\alpha$ Glc1-P content in simultaneous reaction at varied enzyme activity ratios (200 mM sucrose, 50 mM glucose and phosphate, pH 7.0, 45 °C). The *soluble mole ratio* of cellodextrins is the mole ratio of total glucosyl units in the cellodextrins that remained soluble to the glucosyl units transferred from  $\alpha$ Glc1-P in the overall reaction. Enzyme activity ratios (*BaScP*: *CuCbP*: *CcCdP*, U/mL):  $\circ$  reference reaction, 10:3:2;  $\blacklozenge$  10:1:2;  $\blacktriangledown$  10:5:2;  $\blacktriangle$  10:3:1;  $\blacksquare$  10:3:3. The data are from single representative time-course experiments, but agree within typically  $\leq 10\%$  with replicates performed ( $N = 3$ ).



**Supporting Figure S5.** Time-course analysis on sucrose conversion and soluble cellodextrins release in simultaneous reaction (200 mM sucrose, 50 mM glucose, 10 U/mL *BaScP*, 3 U/mL *CuCbP* and 2 U/mL *CcCdP* at 45°C and pH 7.0) at varied phosphate concentrations: **A)** 25 mM and **B)** 80 mM as compared to the reference (50 mM phosphate):  $\bullet$  sucrose (reference in grey),  $\blacksquare$  total soluble cellodextrin (reference in grey),  $\triangle$  cellobiose (G3),  $\times$  cellotetraose (G4),  $+$  cellopentaose (G5),  $\circ$  cellohexaose (G6). The data are from single representative time-course experiments, but agree within typically  $\leq 10\%$  with replicates performed ( $N = 3$ ).

## Reference

- [1] van den Broek, L. A., van Boxtel, E. L., Kievit, R. P., Verhoef, R., Beldman G., Voragen A. G., Physico-chemical and transglucosylation properties of recombinant sucrose phosphorylase from *Bifidobacterium adolescentis* DSM20083. *Appl Microbiol Biotechnol* **2004**, *65*, 219-227.
- [2] Cerdobbel, A., De Winter, K., Aerts, D., Kuipers, R., Joosten H. J., Soetaert W., Desmet T., Increasing the thermostability of sucrose phosphorylase by a combination of sequence- and structure-based mutagenesis. *Protein Eng Des Sel* **2011**, *24*, 829-834.
- [3] Wildberger, P., Luley-Goedl, C., Nidetzky, B., Aromatic interactions at the catalytic subsite of sucrose phosphorylase: their roles in enzymatic glucosyl transfer probed with Phe52-->Ala and Phe52-->Asn mutants. *FEBS Lett* **2011**, *585*, 499-504.
- [4] Alexander, J. K., Characteristics of cellobiose phosphorylase. *J Bacteriol* **1961**, *81*, 903-910.