

Supplementary Materials

Resilient enzymes through immobilisation: Stable NDP polyphosphate phosphotransferase from *Ruegeria pomeroyi* for nucleotide regeneration

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1. Expression and purification of the enzyme

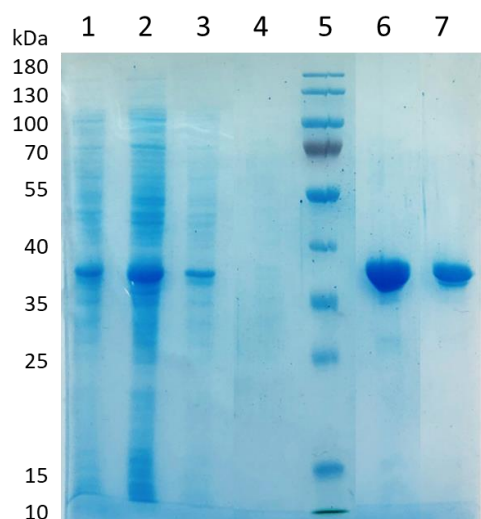


Figure S1: SDS-PAGE of RpPPK2-3 (1: lysate, 2: pellet, 3: lysate after filtration, 4: flow-through, 5: Marker (PageRuler™ Prestained Protein Ladder, 10 to 180 kDa (Thermo Scientific™)), 6: IMAC fraction with RpPPK2-3, 7: retentate after dialysis).

2. Stability of the soluble RpPPK2-3

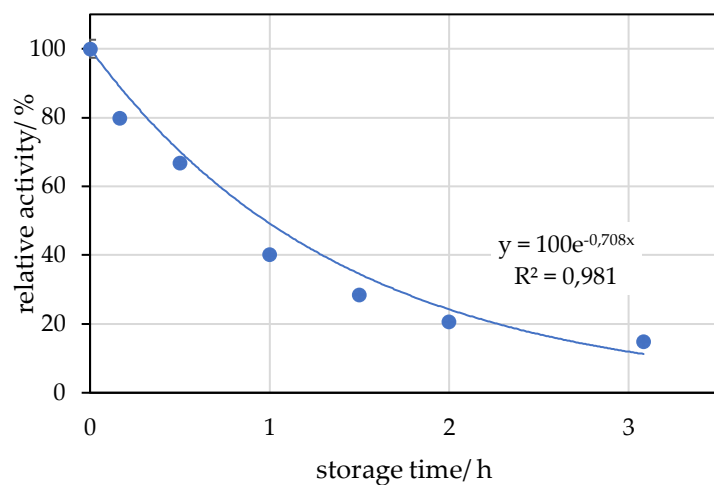


Figure S2: Storage stability study of RpPPK2-3 in Tris buffer (Storage conditions: 22 °C, 40 mg·L⁻¹ RpPPK2-3, 50 mmol·L⁻¹ Tris buffer pH 7.8/ Reaction conditions activity assay: 4 mg·L⁻¹ RpPPK2-3, T= 30 °C, 500 rpm, 50 mmol·L⁻¹ Tris buffer pH 7.8, 30 mmol·L⁻¹ MgCl₂, 7.3 g·L⁻¹ PolyP, 5 mmol·L⁻¹ CDP, V= 1 mL, reaction time: 2.5 min) Error bars show standard deviations of two independent experiments.

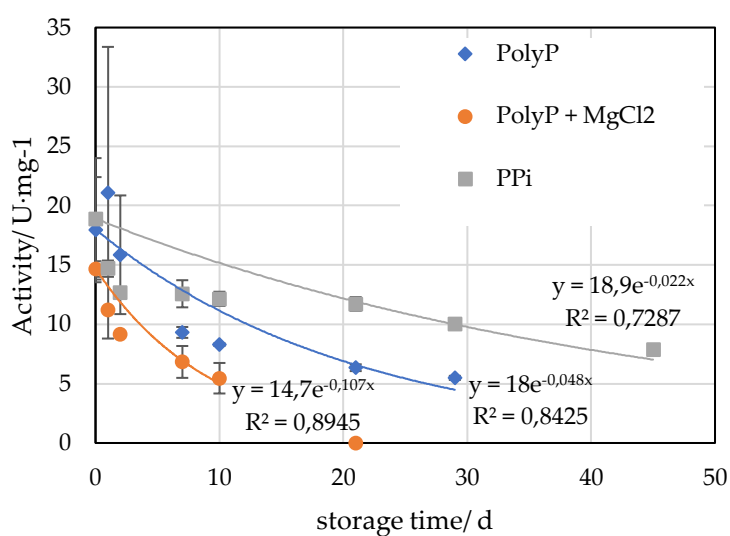


Figure S3: Storage stability study of RpPPK2-3 in Tris buffer with added substrate (Storage conditions: 30 °C, 40 mg·L⁻¹ RpPPK2-3, 50 mmol·L⁻¹ Tris buffer pH 8.0/ Additive concentrations: 10 mmol·L⁻¹ PPi, 8.5 g·L⁻¹ PolyP, 33 mmol·L⁻¹ MgCl₂/ Reaction conditions activity assay: 4 mg·L⁻¹ RpPPK2-3, T= 20 °C, 500 rpm, 50 mmol·L⁻¹ Tris buffer pH 8.0, 30 mmol·L⁻¹ MgCl₂, 7.3 g·L⁻¹ PolyP, 5 mmol·L⁻¹ CDP, V= 1 mL, reaction time: 2.5 min) Error bars show standard deviations of two independent experiments.

3. Stability of the immobilised RpPPK2-3

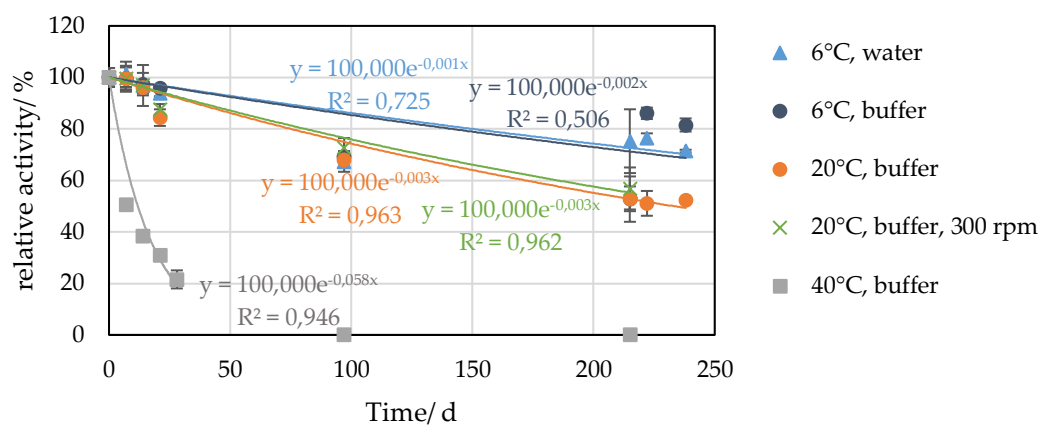


Figure S4: Stability study of RpPPK2-3 immobilised on epoxy methacrylate ECR8209M (Storage conditions as described in the legend/ Reaction conditions activity assay: 5 mmol·L⁻¹ CDP, 7.3 g·L⁻¹ PolyP, 30 mmol·L⁻¹ MgCl₂, 50 mmol·L⁻¹ Tris pH 7.8, T= 20 °C, 1000 rpm, V= 1.5 mL, 7 g·L⁻¹ immobilisate, reaction time= 15 min) Error bars show standard deviations of two independent experiments.

4. Activity assay

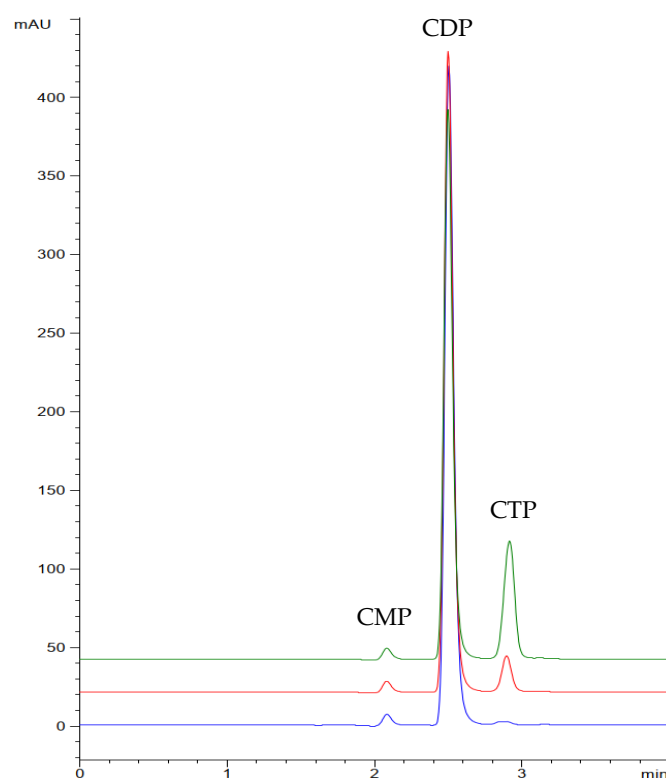


Figure S5: HPLC chromatogram of samples taken at different times in the activity assay (blue: substrate, red: sample after 5 min reaction, green: sample after 30 min reaction/ Reaction conditions activity assay: 5 mmol·L⁻¹ CDP, 7.3 g·L⁻¹ PolyP, 30 mmol·L⁻¹ MgCl₂, 50 mmol·L⁻¹ Tris pH 7.8, T= 20 °C, 500 rpm, V= 1 mL, 4 mg·L⁻¹ immobilisate, reaction time= 5 and 30 min/ HPLC conditions: Phenomenex Luna 3u C18(2) 100 Å (150 X 4.6 mm, 3 µm particles) column, 40 °C, 0.8 mL·min⁻¹, 46 % acetonitrile, 54 % 20 mmol·L⁻¹ potassium phosphate buffer containing 20 mmol·L⁻¹ tetrabutylammonium bromide (TBAB) at pH 5.9, detection at 272 nm (VWD))