

Supplementary materials

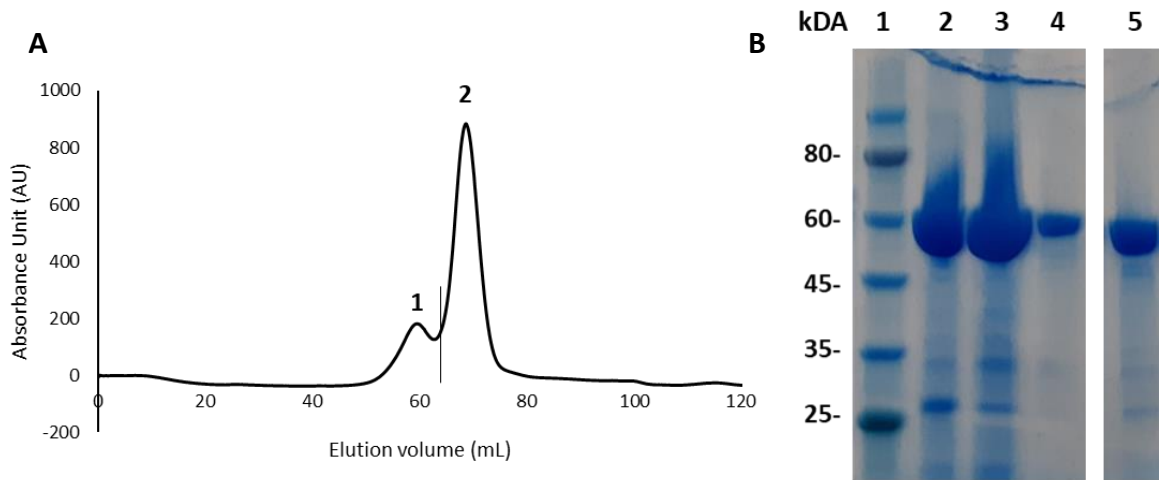


Figure S1: A) Size exclusion chromatography elution profile (Superdex 200 HiLoad 16/600) of cDPGS. A first peak 1 eluting at 60 mL corresponds to cDPGS with an oligomeric state for a suggested tetramer, while the second peak 2 eluting at 70 mL is cDPGS with an apparent molecular mass corresponding to a dimeric state of the protein (~100 kDa). A sample from peak 2 was used for the crystallization studies. B) SDS-PAGE showing the purification of cDPGS. Lane 1: Molecular weight markers, lane 2 and 3: Ni-NTA elution, lane 4: SEC Peak 1 and lane 5: SEC Peak 2.

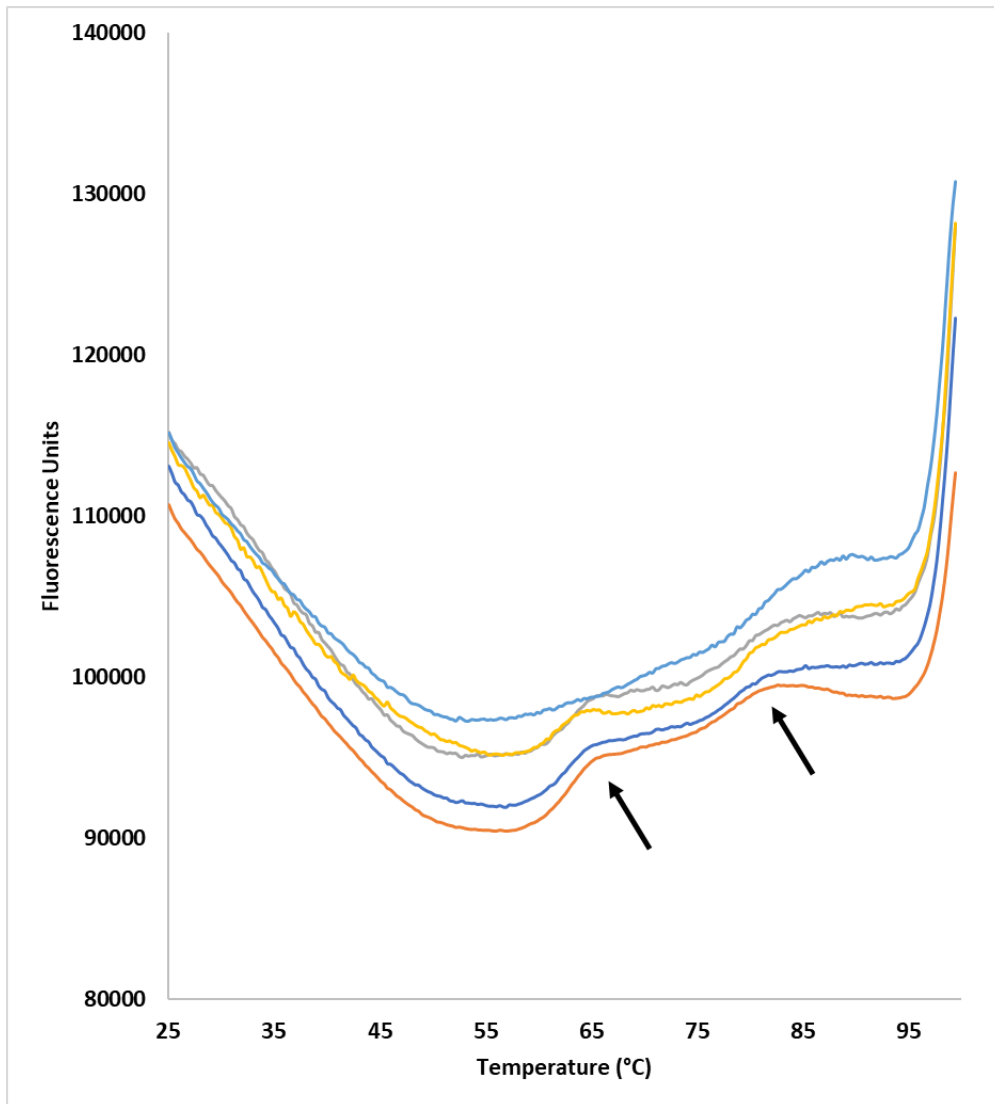


Figure S2: Results of differential scanning fluorimetry of cDPGS. Five replicate traces are shown. The fluorophore used (SYPRO Orange) is temperature sensitive and so the fluorescence initially drops with temperature. Minor fold relaxation events occur as indicated by the arrows around 70 °C and 85 °C. These suggest a conformational change of the protein structure to facilitate activity as often observed for thermophilic enzymes. The overall protein unfolding event is initiating between 95 °C and 99 °C (the temperature limit of the instrument) but has clearly not reached a plateau.

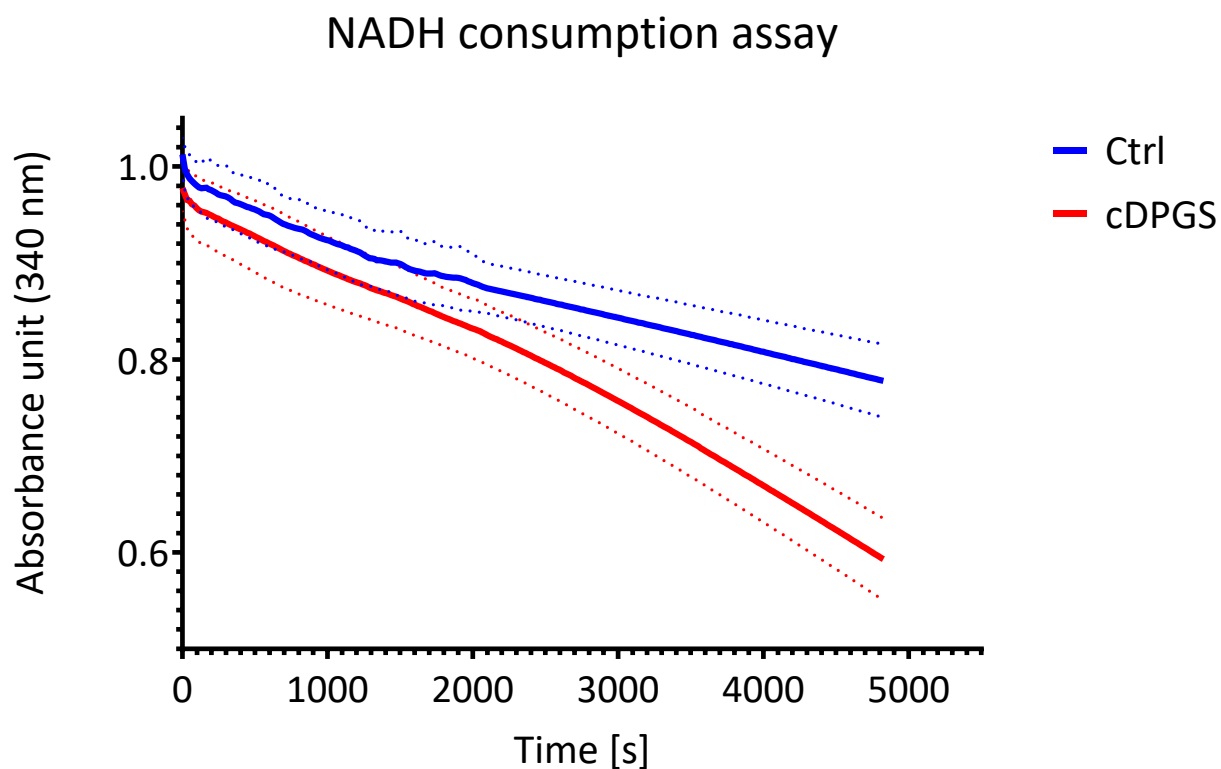


Figure S3: Coupled assay following the consumption of 2,3 DPG. The cDPGS activity was indirectly observed through the amounts of ADP produced from ATP calculated from the respective reactions coupled to the oxidation of NADH using the pyruvate kinase (PK) and lactic acid dehydrogenase (L-LDH) from rabbit muscle. Assays were performed in triplicate and a control reaction was carried out without the enzyme cDPGS, where the total volume was maintained by adding buffer. A background absorbance was observed due to oxidative activity within the assay solution. The error bars are shown as dotted lines.

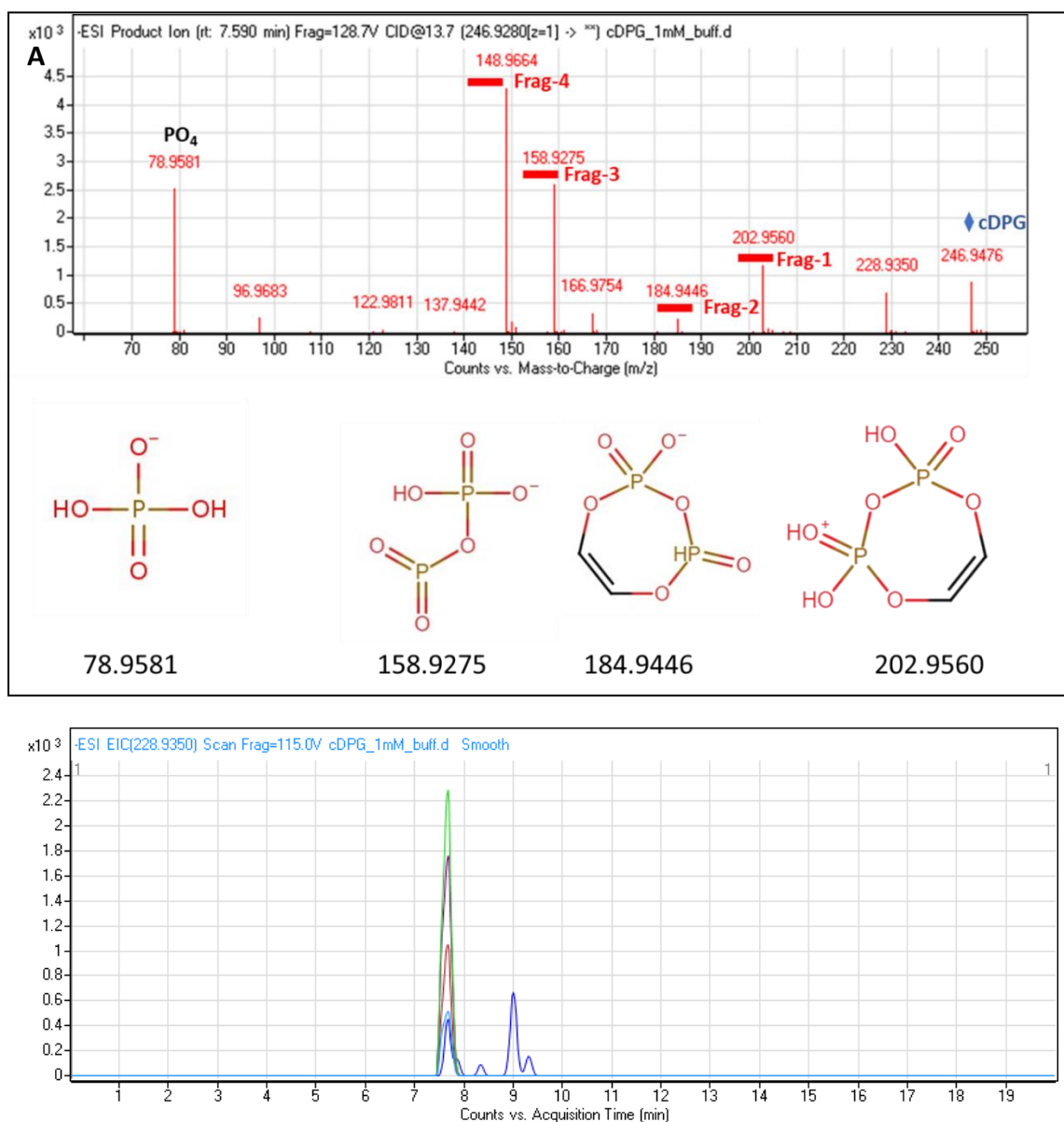


Figure S4: **A**) The MS fragmentation of cDPG, the whole molecule mass (cDPG: 246.9 Da) is indicated by the blue diamond, while the four fragments' masses are underlined in red, the structures of three of the fragments were predicted with CFM-ID and are shown. **B**) Extracted chromatogram of the reaction products at the rt of the cDPG. Green 202.9560, Purple 148.9664, Brown 158.9275, Light Blue 184.9446, Dark blue 78.9581. The structure of the fragment with mass 148.9664 could not be determined.

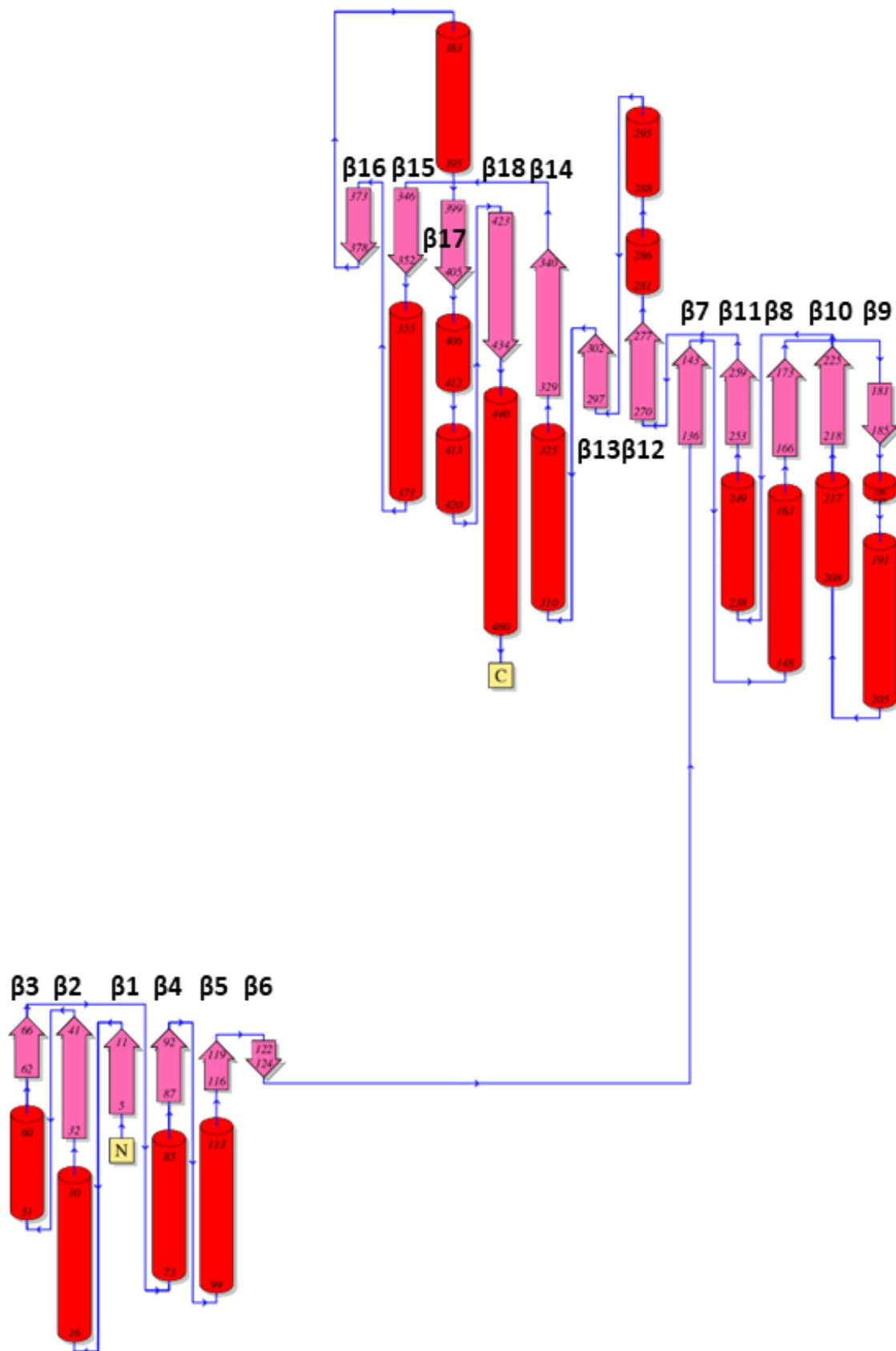


Figure S5: A secondary structure topology diagram of the cDPGS enzyme prepared using LigPlot (Laskowski and Swindells, 2011)

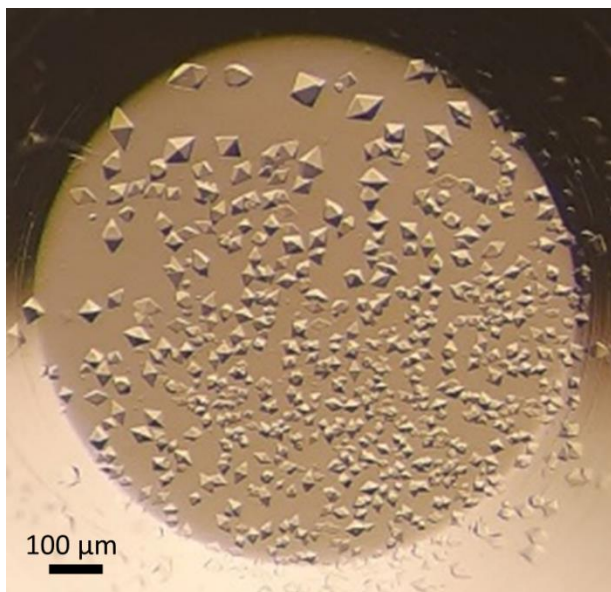


Figure S6: The crystals obtained for the complex with cDPG substrate and ADP bound crystals in condition E12 of the Morpheus™ screen.

Figure S7: Nucleotide sequence of the pLATE51/cDPGS plasmid

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pLATE51-cDPGS.gb

GenBank file of the pLATE51/cDPGS plasmid: