

Supplementary Information for

**Structural comparison of a promiscuous and a highly specific
sucrose 6^F-phosphate phosphorylase**

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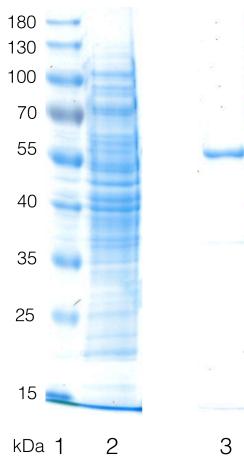


Figure S1 SDS-PAGE for samples of IcSPP. (1) PageRuler prestained protein ladder (ThermoFisher), (2) cell-free extract, (3) purified fraction.

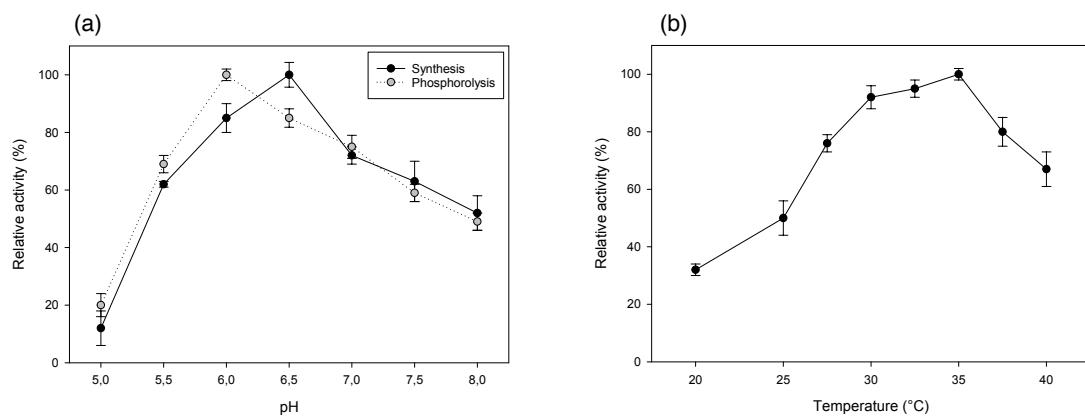


Figure S2 The effect of (a) pH and (b) temperature on IcSPP activity. The pH profile was determined in the synthesis (black circles) and the phosphorolytic (grey circles) directions; the temperature profile was determined in the synthesis direction.

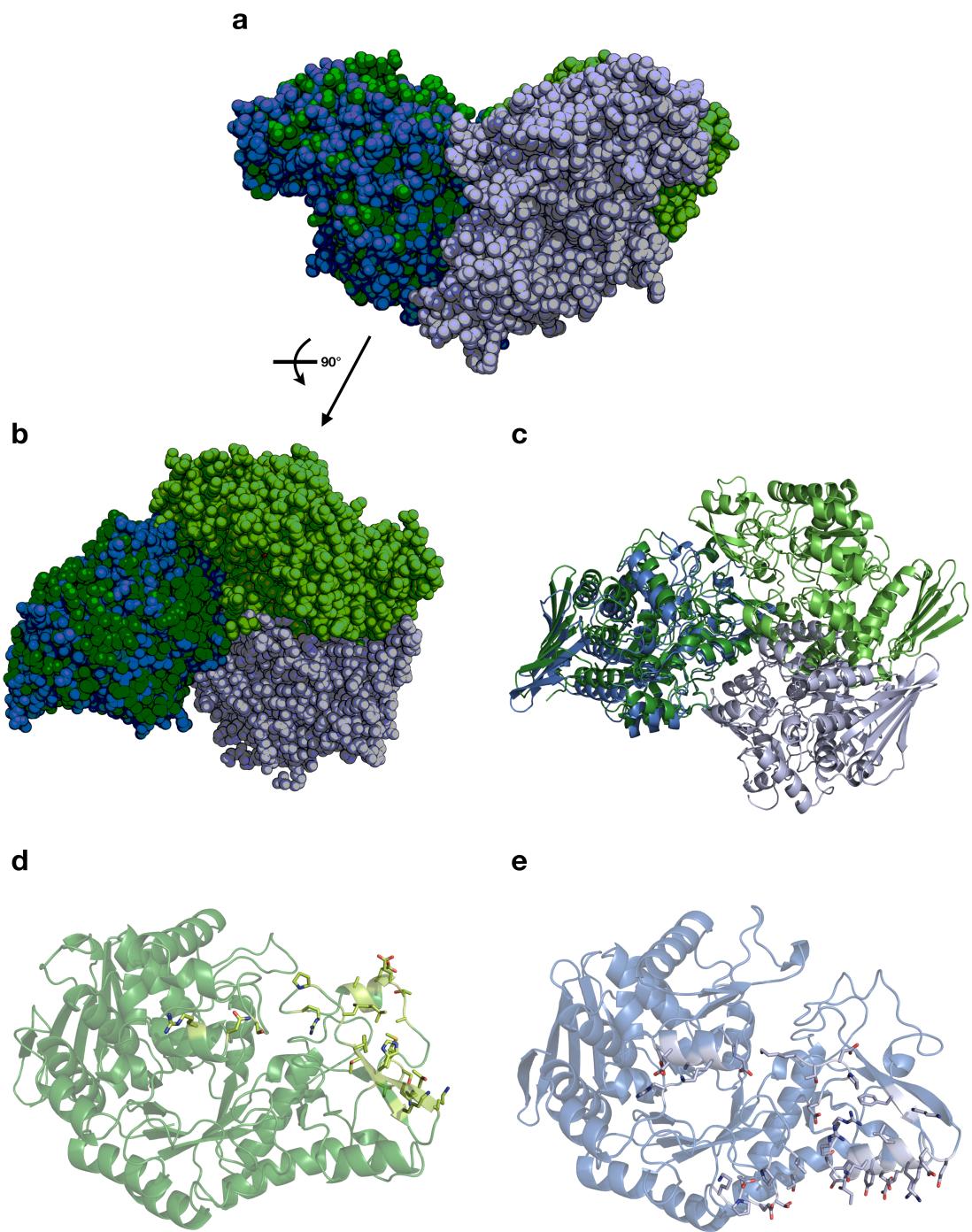


Figure S3 The BaSP (green) and TtSPP (blue) homodimeric structures with molecule B superposed, shown as spheres (a, b) or in cartoon representation (c). One monomer of BaSP (d) and TtSPP (e) with the residues at the dimer interface shown as sticks.

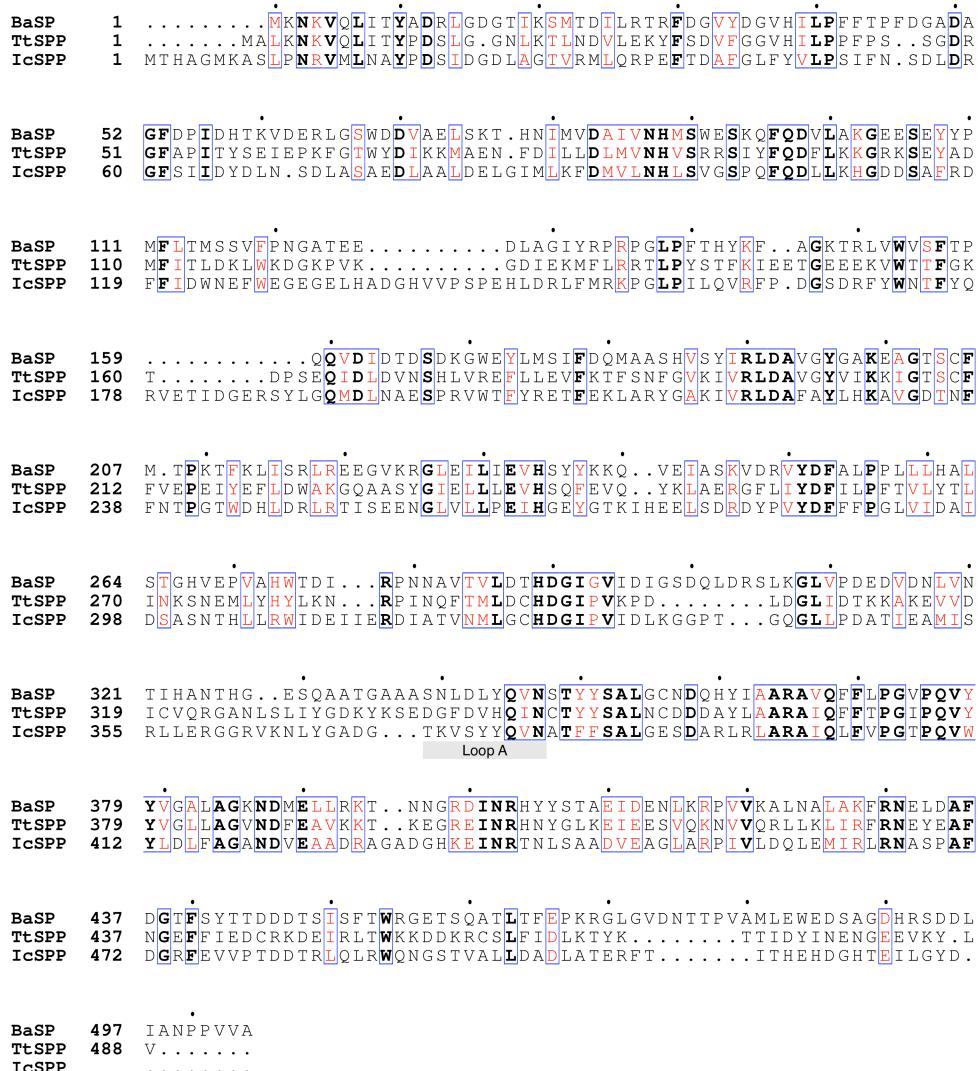


Figure S4 Multiple sequence alignment for BaSP, TtSPP and IcSPP. A position is framed in blue if the majority of its residues are similar. Identical residues are shown in bold, similar residues are shown in red. Loop A is indicated with a grey bar below the sequence. The alignment was visualised using ESPript 3.0 (<http://escript.ibcp.fr>).

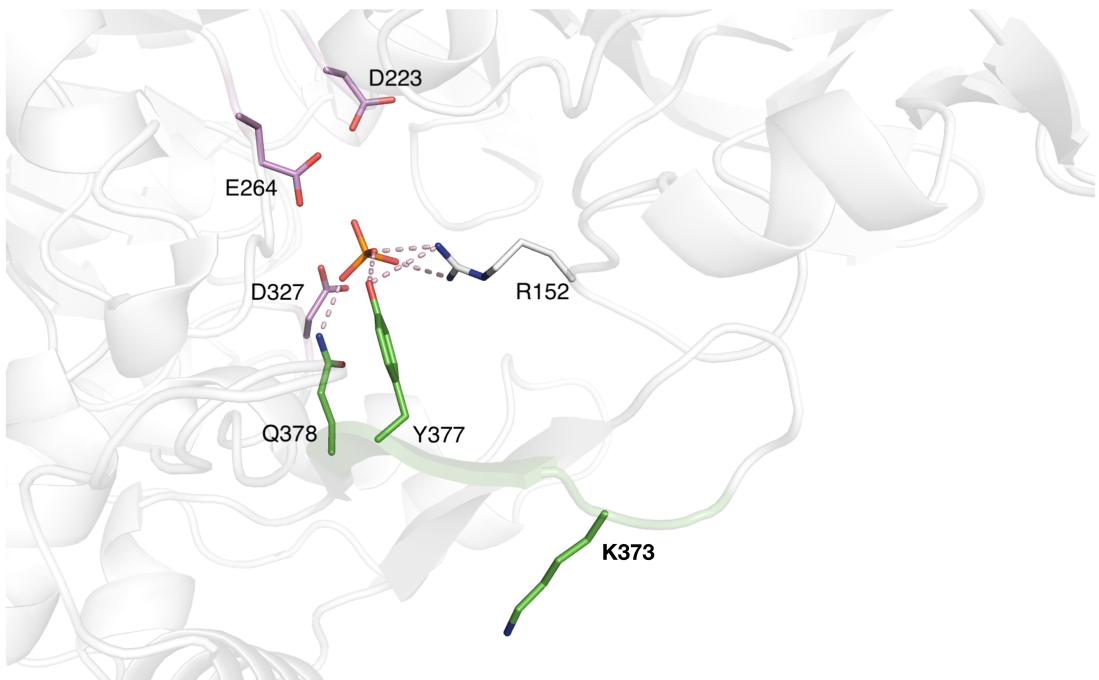


Figure S5 Position of the conserved residue Lys373 in the crystal structure of IcSPP. Shown for reference: catalytic residues (purple), bound phosphate ion and the residues binding the phosphate ion. Loop A is colored in green.

Table S1 Compounds evaluated as substrates for IcSPP.

Reaction	Substrate
Phosphorolysis	Sucrose
	Sucrose 6 ^F -phosphate
	2-O-Glucosylglycerol
	2-O-Glucosylglycerate
Synthesis	D-Glucose
	D-Mannose
	D-Galactose
	D-Fructose
	D-Tagatose
	D-Xylose
	D-Ribose
	D-Arabinose
	D-Psicose
	L-Sorbose
	L-Fucose
	L-Arabinose
	L-Rhamnose
	L-Xylose
	L-Ribose
	Gluconic acid
	Glucuronic acid
	Galacturonic acid
	Glucosamine
	Galactosamine
	N-Acetyl-D-Glucosamine
	N-Acetyl-D-Galactosamine
	D-Glyceric Acid
	Glycerol
	D-Mannitol
	D-Sorbitol
	D-Xylitol
	D-Ribitol
	Erythritol
	Trehalose
	Kojibiose
	Maltose
	Sophorose
	Cellobiose
	Sucrose

Table S2 Data collection and refinement statistics.

	IcSPP	TtSPP
Data collection		
PDB code	6S9U	6S9V
Beamline	ESRF ID30A-3	ESRF ID30A-3
Wavelength (Å)	0.9677	0.9677
Space group	C222 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cell (Å)	63.4, 92.1, 180.0	79.3, 83.7, 147.4
Resolution (Å)	46 – 2.05 (2.11 – 2.05)	38 – 1.83 (1.86 – 1.83)
Total reflections	169930	461327
Unique reflections	33373	86925
Completeness (%)	99.6 (100.0)	99.8 (99.8)
Redundancy	5.1 (5.1)	5.3 (5.5)
Mean $I/\sigma(I)$	9.7 (2.1)	12.7 (2.3)
R _{merge}	0.096 (0.749)	0.068 (0.605)
CC _{1/2}	0.995 (0.638)	0.998 (0.801)
Refinement		
non-H atoms AU		
Protein, solvent, other	4141, 313, 51	8075, 464, 117
R_{work} , R_{free}	0.169, 0.202	0.158, 0.184
r.m.s.d. from ideal values		
Bond lengths (Å)	0.005	0.005
Bond angles (°)	0.7	0.8
B-factors (Å ²)		
Protein, solvent, other	30.0, 32.6, 48.8	30.8, 33.9, 58.8
Ramachandran plot (%)		
Favored, allowed, outliers	97.1, 2.7, 0.2	97.3, 2.7, 0.0
Ramachandran plot (%)		
Favored, allowed, outliers	97.1, 2.7, 0.2	97.3, 2.7, 0.0

Highest resolution shell in parenthesis. AU is asymmetric unit.

Table S3 Protein sequences for the sucrose 6'-phosphate phosphorylases from *Illumatobacter coccineus* (IcSPP) and from *Thermoanaerobacterium thermosaccharolyticum* (TtSPP). The His₆-tag and linker sequences are underlined.

Enzyme	Sequence
IcSPP	MTHAGMKASLPNRMVNAYPDSIDGDLAGTVRMLQRPEFTDAFGLFY VLPSIFNSDLDRGFSIIDYDLSNLASAEDLAALDELGIMLKFDML NHLSVGSPQFQDLLKHGDDSAFRDFFIDWNEFWEGEGELHADGHVVP SPEHLDRLFLMRKPGPLPILOQVRFPDGSDRFYWNTFYQRVETIDGERSY LGQMDLNAESPRVWTFYRETFEKLARYGAKIVRLDAFAYLHKAVGDT NFFNTPGTWDHLDRLRTISEENGLVLLPEIHGEYGTKIHEELSDRDY PVYDFFFPGGLVIDAIDSASANTHLLRWIDEIIERDIATVNMLGCHDGI PVIDLKGGPTGQGLLPDATIEAMISRLLERGGRVKNLYGADGTKVSY YQVNATFFSALGESDARLRLARAQLFVPGTPQVWYLDLFAGANDVE AADRAGADGHKEINRTNLSAADVEAGLARPIVLDQLEMIRLRNASPA FDGRFEVVPTDDTRLQLRWQNGSTVALLDADLATERFTITHEHDGHT EILGYDLEHHHHHH
TtSPP	<u>MGGSHHHHHHGMASMALKNVQLITYPDSDLGGNLKTLNDVLEKYFSD</u> VFGGVHILPPFPSSGDRGFAPITYSEIEPKFGTWYDIKKMAENFDIL LDLMVNHSRRSIYFQDFLKKGRKSEYADMFITLDKLWKDGKPVKGDI IEKMFLRRTLPHYSTFKIEETGEEEKVWTTFGKTDPSEQIDLDVNSHL VREFLLEVFKTFSNFGVKIVRLDAVGYVIKKIGTSCFFVEPEIYEFL DWAKGQAASYGIELLLEVHSQFEVQYKLAERGFLIYDFILPFTVLYT LINKSNEMLYHYLKKNRPINQFTMLDCHDGIPVKPDLDGLIDTKKAKE VVDICVQORGANLSLIYGDKYKSEDGFDVHQINCTYSALNCDDAYL AARAIQFFTPGIPQVYYVGLLAGVNDFEAVKKTKEGREINRHNYGLK EIEESVQKNNVQRLKLIRFRNEYEAFNGEFFIEDCRKDEIRLTWKK DDKRCSLFIDLKYKTTIDYINENGEEVKYLV