Supporting Information

Assessing the role of deep eutectic solvents in *Yarrowia lipolytica* inhibition

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Table S1: *Yarrowia lipolytica* microbial growth in the presence of deep eutectic solvents based on ammonium salts and fatty acids at 20 wt% concentration[a].

Hydrogen bond acceptor	Hydrogen bond donor	Microbial growth (g dry cell weight L^{-1})	pH
$[N_{8881}]Cl$	Hexanoic acid	$11.95^{b} \pm 0.60$	$7.08^{a} \pm 0.09$
	Octanoic acid	$5.16^{b,c} \pm 0.13$	$7.19^{a} \pm 0.11$
	Decanoic acid	$2.88^{c,d} \pm 0.09$	$7.12^{a} \pm 0.05$
$[N_{4444}]Cl$	Hexanoic acid	$5.77^{b} \pm 0.24$	$7.11^{a} \pm 0.07$
	Octanoic acid	$3.20^{b,c} \pm 0.06$	$7.14^{a} \pm 0.08$
	Decanoic acid	$2.25^{c,d} \pm 0.19$	$7.13^{a} \pm 0.07$
ChCl	Hexanoic acid	$6.09^{b} \pm 0.47$	$7.09^{a} \pm 0.09$
	Octanoic acid	$2.06^{b,c} \pm 0.39$	$7.14^{a} \pm 0.10$
	Decanoic acid	$1.75^{c} \pm 0.15$	$7.09^{a} \pm 0.11$
$[N_{8888}]Br$	Hexanoic acid	$2.18^{b} \pm 0.27$	$7.11^{a} \pm 0.08$
	Octanoic acid	$1.03^{b,c} \pm 0.11$	$7.14^{a} \pm 0.07$
	Decanoic acid	$0.68^{c} \pm 0.17$	$7.12^{a} \pm 0.09$
$[N_{8881}]Br$	Hexanoic acid	$5.28^{b} \pm 0.22$	$7.13^{a} \pm 0.10$
	Octanoic acid	$3.26^{b,c} \pm 0.13$	$7.08^{a} \pm 0.08$
	Decanoic acid	$1.89^{c,d} \pm 0.15$	$7.08^{a} \pm 0.09$
$[N_{4444}]Br$	Hexanoic acid	$3.90^{b} \pm 0.22$	$7.11^{a} \pm 0.11$
	Octanoic acid	$2.30^{b,c} \pm 0.25$	$7.12^{a} \pm 0.12$
	Decanoic acid	$1.28^{c,d} \pm 0.34$	$7.10^{a} \pm 0.09$

[a] Control showed a microbial growth of $13.12^{a} \pm 0.64$ g dry cell weight L^{-1} and pH of $7.20^{a} \pm 0.11$. Means with different lowercase letters in the same column are significantly different ($p \leq 0.05$).

Table S2: Transport energy features between ligands (HBA, HBD, subtract, and products) and the most favorable enzyme tunnels towards the active site.

Ligand	Tunnel	E_{bound}	E_{max}	$E_{surface}$	E_a	ΔE_{BS}
$[N_{8888}]^+$	1	-4.9	-3.9	-4.0	0.1	-0.9
	2	-4.7	-3.0	-3.3	0.3	-1.4
	3	-4.4	-2.7	-2.7	0.0	-1.7
	4	-4.4	-3.2	-3.5	0.3	-0.9
$[N_{8881}]^+$	1	-5.0	-3.1	-4.1	0.2	-0.9
	2	-4.6	-3.9	-3.1	0.0	-1.5
	3	-4.8	-2.7	-2.8	0.1	-2.0
	4	-4.5	-3.0	-3.4	0.4	-1.1
$[N_{4444}]^+$	1	-3.9	-3.1	-3.1	0.0	-0.8
	2	-4.2	-2.6	-2.6	0.0	-1.6
	3	-4.2	-2.2	-2.2	0.0	-2.0
	4	-4.3	-2.4	-2.6	0.2	-1.7
$[Ch]^+$	1	-2.4	-1.8	-1.8	0.0	-0.6
	2	-2.5	-2.0	-2.1	0.1	-0.4
	3	-2.4	-2.0	-2.3	0.3	-0.1
	4	-2.5	-1.7	-1.7	0.0	-0.8
Hexanoic acid	1	-3.1	-3.1	-2.8	0.0	-0.3
	2	-3.6	-2.7	-3.2	0.1	-0.4
	3	-3.1	-2.5	-2.5	0.0	-0.6
	4	-3.1	-2.0	-2.0	0.0	-1.1
Octanoic acid	1	-3.5	-3.0	-3.1	0.1	-0.4
	2	-3.5	-3.0	-3.1	0.1	-0.4
	3	-3.3	-2.4	-2.4	0.0	-0.9
	4	-3.4	-2.4	-2.5	0.1	-0.9
Decanoic acid	1	-3.9	-3.1	-3.1	0.0	-0.8
	2	-3.4	-3.0	-3.0	0.0	-0.4
	3	-3.5	-2.5	-2.8	0.3	-0.7
	4	-3.3	-2.5	-2.6	0.1	-0.7
Glucose	1	-4.2	-3.4	-3.6	0.2	-0.6
	2	-4.7	-3.5	-3.8	0.1	-0.9
	3	-4.4	-3.5	-4.2	0.7	-0.2
	4	-4.3	-2.7	-2.7	0.2	-1.6

Table S3: Transport energy features between ligands (HBA, HBD, subtract, and products) and the most favorable enzyme tunnels towards the surface.

Ligand	Tunnel	E_{bound}	E_{max}	$E_{surface}$	E_a	ΔE_{BS}
$[N_{8888}]^+$	1	-3.6	-3.6	-4.4	0.0	0.8
	2	-2.7	-2.7	-4.7	0.0	2.0
	3	-2.7	-2.4	-4.7	0.3	2.0
	4	-2.8	-2.8	-4.4	0.0	1.6
$[N_{8881}]^+$	1	-3.4	-3.1	-4.7	0.3	1.3
	2	-2.4	-2.4	-4.7	0.0	2.3
	3	-2.6	-2.2	-4.7	0.4	2.1
	4	-2.9	-2.8	-4.4	0.1	1.5
$[N_{4444}]^+$	1	-2.8	-2.7	-4.2	0.1	1.4
	2	-2.2	-2.2	-4.1	0.0	1.9
	3	-1.9	-1.9	-4.1	0.0	2.2
	4	-2.4	-2.3	-4.0	0.1	1.6
$[Ch]^+$	1	-1.5	-1.5	-2.5	0.0	1.0
	2	-2.1	-2.1	-2.5	0.0	0.4
	3	-1.4	-1.4	-2.5	0.0	1.1
	4	-1.6	-1.3	-2.6	0.3	1.0
Hexanoic acid	1	-2.3	-2.3	-3.0	0.0	0.7
	2	-2.3	-2.3	-3.3	0.0	1.0
	3	-2.1	-2.1	-3.2	0.0	1.1
	4	-2.4	-1.8	-3.1	0.6	0.7
Octanoic acid	1	-2.8	-2.8	-3.3	0.0	0.5
	2	-2.5	-2.5	-3.4	0.0	0.9
	3	-2.2	-2.1	-3.5	0.1	1.3
	4	-2.2	-2.2	-3.3	0.0	1.1
Decanoic acid	1	-2.9	-2.9	-3.6	0.0	0.7
	2	-2.6	-2.6	-3.6	0.0	1.0
	3	-2.4	-2.1	-3.7	0.3	1.3
	4	-2.3	-2.3	-3.4	0.0	1.1
G6P	1	-2.8	-3.6	-4.9	0.3	1.3
	2	-3.6	-2.5	-4.3	0.0	1.5
	3	-3.0	-2.7	-4.7	0.3	1.7
	4	-2.5	-2.5	-4.6	0.0	2.1

Table S4: Docking affinity energy and interacting atoms predicted by AutoDock vinna between ligands (HBA, HBD, subtract, and products) and amino acids[a].

Ligands	Affinity (kcal·mol^{-1})	Binding types	Amino acids (AA)	From	To	Distance (Å)
$[N_{8888}]^+$	-5.3	Electrostatic	**GLU338**	ligand	AA	3.40
		Hydrophobic	PRO330			4.56
			ARG331			3.97
			ILE267			4.71
			LYS212	AA	ligand	4.65
			ARG331			4.92
			TYR344			5.44
		van der Waals	THR211			
			LYS212			
			ASN246			
			ASP247			
			THR270			
			GLY271			
			VAL272			
			GLU305			
			SER329			
			GLN335			
			LYS339			
			TYR345			
$[N_{8881}]^+$	-5.4	Electrostatic	**GLU338**	ligand	AA	3.33
		Hydrogen Bond	**GLU338**			3.73
		Hydrophobic	TYR344			3.71
			ARG331			4.08
			ILE267			4.69
			PRO330	AA	ligand	3.93
			ARG331			4.37
			TYR344			5.38
		van der Waals	SER194			
			PRO196			
			THR211			
			LYS212			
			THR270			
			GLY271			
			VAL272			
			ASN273			
			GLU305			
			SER329			
			GLN334			
			GLN335			
			LYS339			
			TYR345			
$[N_{4444}]^+$	-4.5	Electrostatic	**GLU338**	ligand	AA	4.67

		Hydrogen Bond	**GLU338**			3.49
		Hydrophobic	TYR344			3.58
		van der Waals	THR211 LYS212 **THR270** SER329 PRO330 ARG331 GLN335 LYS339 TYR345			
$[Ch]^+$	-3.5	Electrostatic	**ASP247** **GLU338**	ligand	AA	4.77 4.72
		Hydrogen Bond	**ASP247** **GLU338**			3.65 3.75
		van der Waals	SER194 LYS212 THR248 ILE267 GLY271 VAL272 **ASN273** ASN303 GLU305			
Br^-	-1.1	-	-	-	-	-
Cl^-	-1.2	-	-	-	-	-
Hexanoic acid	-3.4	Hydrogen Bond	GLY502 SER503	AA	ligand	2.35 1.92
			GLY502 ASP460	ligand	AA	2.90 2.37
		Hydrophobic	ILE267			4.32
		van der Waals	ARG93 THR251 **ASP273** GLY461 ASP501 GLY504			
Octanoic acid	-3.8	Hydrogen Bond	**ASN246** **THR270**	AA	ligand	1.97 2.02
		Hydrophobic	PRO196			4.17
		van der Waals	SER194 TYR195 THR211 LYS212 **ASP247** THR248 ILE267			

			GLY271			
			GLU305			
			GLN335			
			GLU338			
Decanoic acid	-4.4	Hydrogen Bond	PRO188			2.57
		Hydrophobic	CYS81			4.59
			ALA159			4.22
			ALA160	AA	ligand	4.29
			TYR82			4.75
			TYR82			4.70
			PHE163			5.35
			ILE515	ligand	AA	5.38
		van der Waals	GLY80			
			VAL155			
			ASP156			
			THR164			
			LYS186			
			ILE187			
			LYS519			
Glucose	-	Hydrogen Bond	**ASN273**	ligand	AA	2.62
			GLY271			2.80
			GLY269	AA	ligand	2.60
			ALA274			2.37
		van der Waals	GLY265			
			PHE268			
			ASP460			
			GLY461			
G6P	-	Hydrogen Bond	ILE267	AA	ligand	2.20
		van der Waals	SER194			
			THR248			
			MET252			
			GLY265			
			PHE268			
			ASN273			
			GLY458			
			GLY461			
			ALA499			
			GLY502			
			SER503			

[a] Bold amino acids are localized in active site.

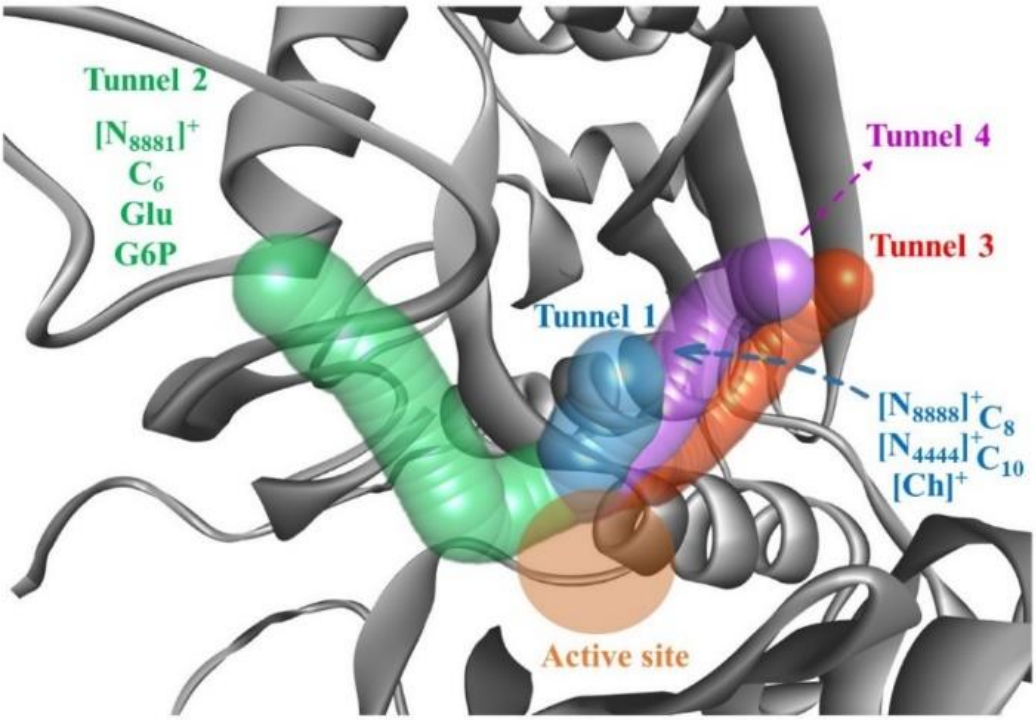

Figure S1: Transport of HBA/HBD, substrate, and products through the *Yarrowia lipolytica* hexokinase tunnels to the active site: tunnel 1 (blue), tunnel 2 (green), tunnel 3 (red), and tunnel 4 (purple).

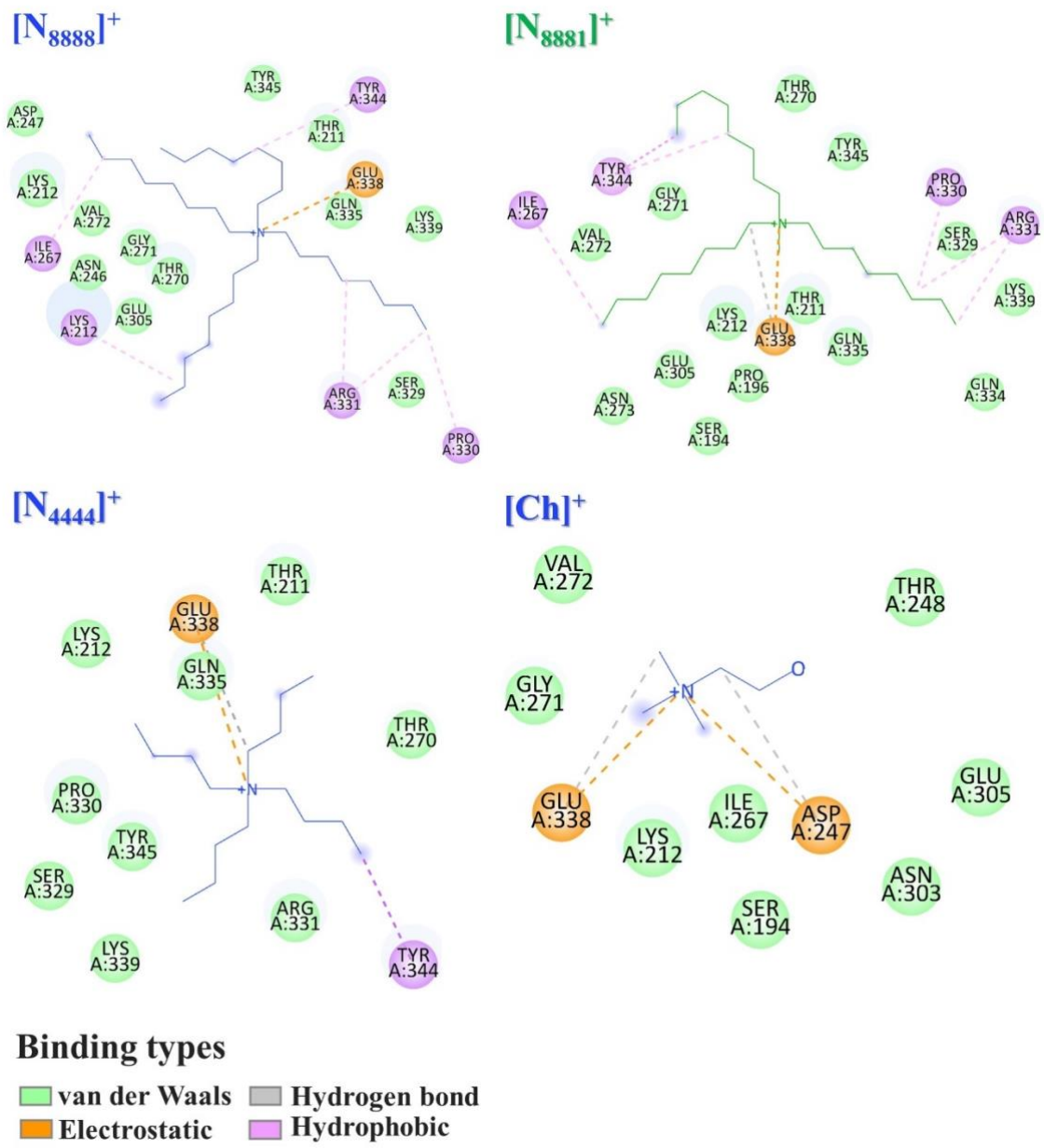

Figure S2: Molecular interaction diagrams between receptor (hexokinase from *Yarrowia lipolytica*) and ligand (HBA-forming cations).

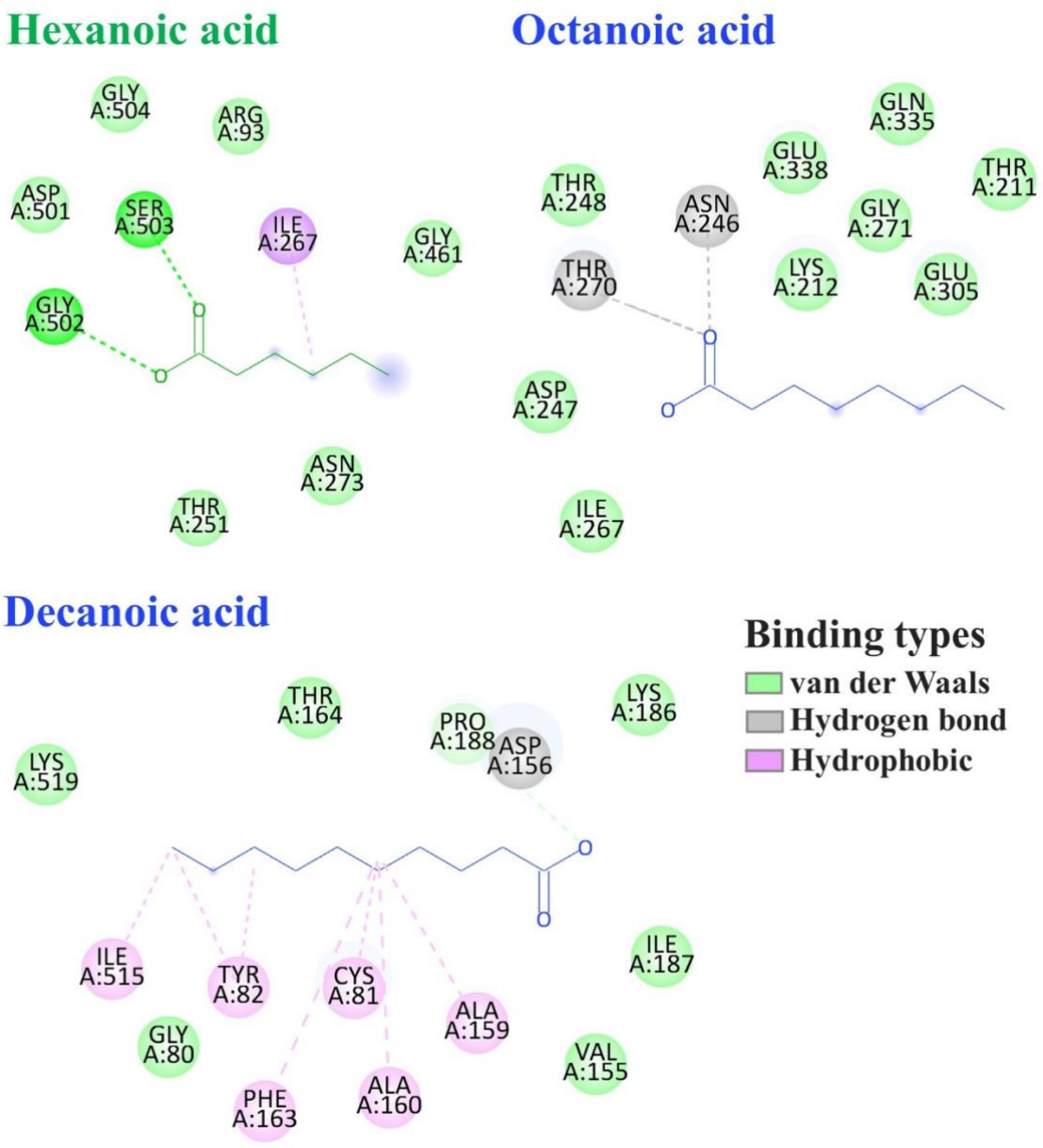

Figure S3: Molecular interaction diagrams between receptor (hexokinase from *Yarrowia lipolytica*) and ligand (HBA – fatty acids).

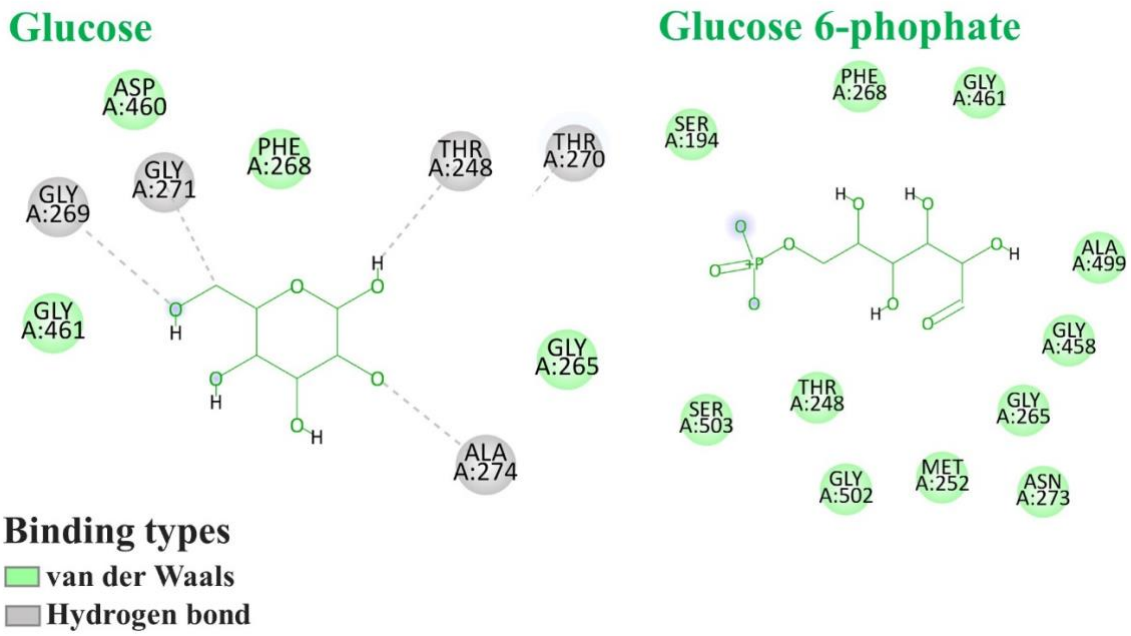

Figure S4: Molecular interaction diagrams between receptor (hexokinase from *Yarrowia lipolytica*) and ligand (substrate and product).

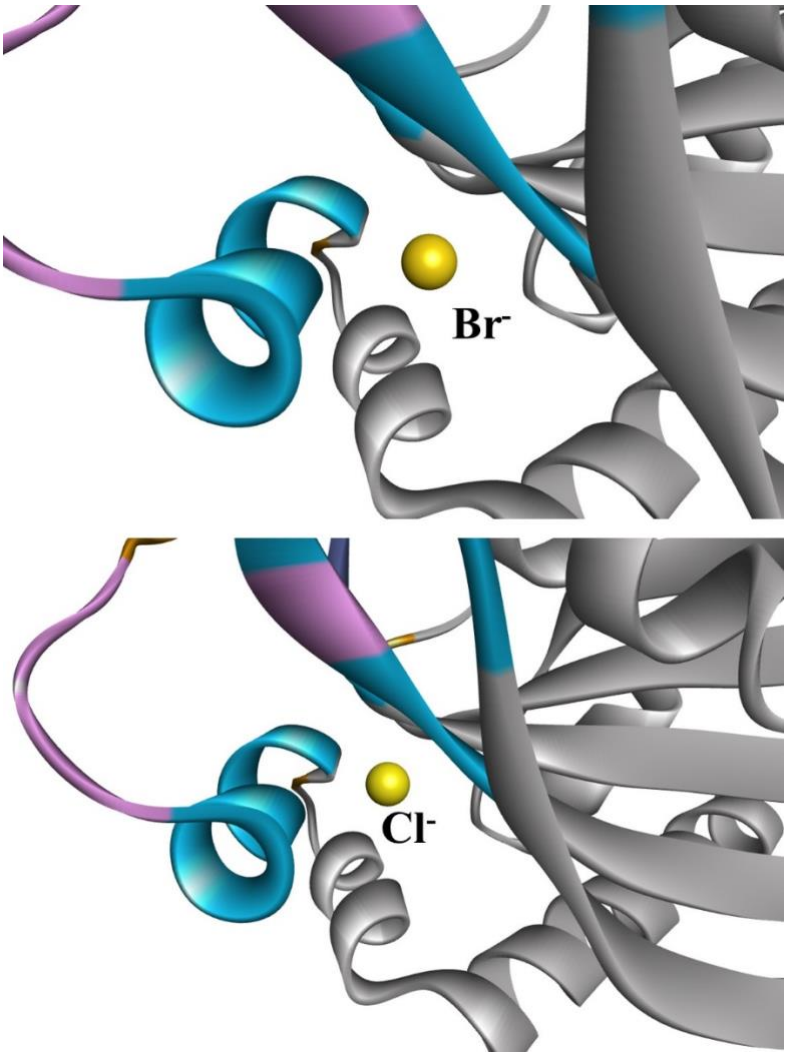

Figure S5: Docking pose with the lowest absolute value of affinity (kcal/mol) between the HBA-forming anion (yellow) and hexokinase allosteric site 1 (blue).