Electronic Supplementary Information

Long-term protein packaging in cholinium-based ionic liquids: Improved catalytic activity and enhanced stability of cytochrome C against multiple stresses

Meena Bisht,[a,b] Dibyendu Mondal,[b,c] Matheus M. Pereira,[b] Mara G. Freire,[b] P. Venkatesu,[a]* and J. A. P. Coutinho[b]*

[a]*Department of Chemistry, University of Delhi, Delhi 110 007, India, E-mail: venkatesup@hotmail.com, pvenkatesu@chemistry.du.ac.in; Fax: +91-11-2766 6605; Tel: +91-11-27666646-142*
[b]*Chemistry Department, CICECO-Aveiro Institute of Materials, University of Aveiro, 3810-193, Aveiro, Portugal. E-mail: jcoutinho@ua.pt; quijorge@ua.pt; Fax: +351 234 370 084; Tel: +351 234 401 507*
[c]Center for Nano and Material Science, Jain University, Bangalore, Karnataka, India-562112
*Corresponding authors

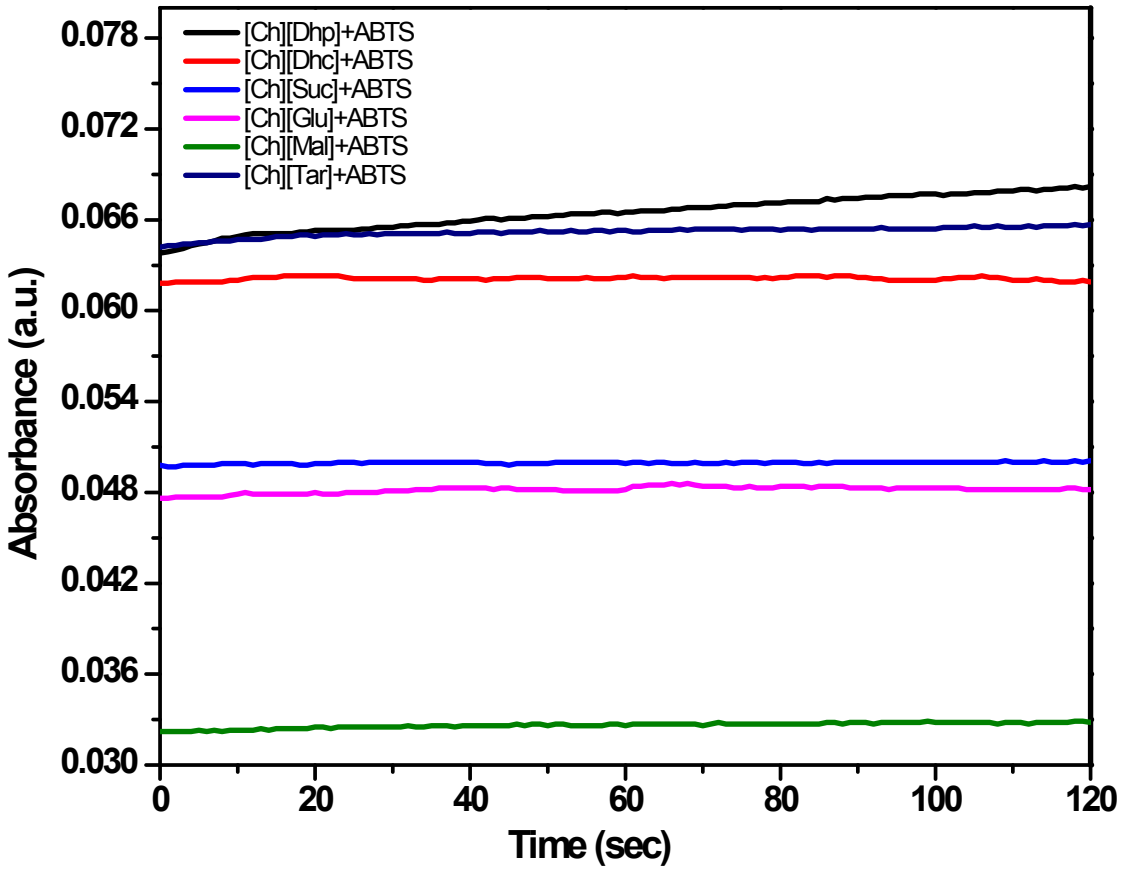

Fig. S1 Background interference of aqueous ILs (IL and water in a 1:2 ratio by weight) and ABTS.

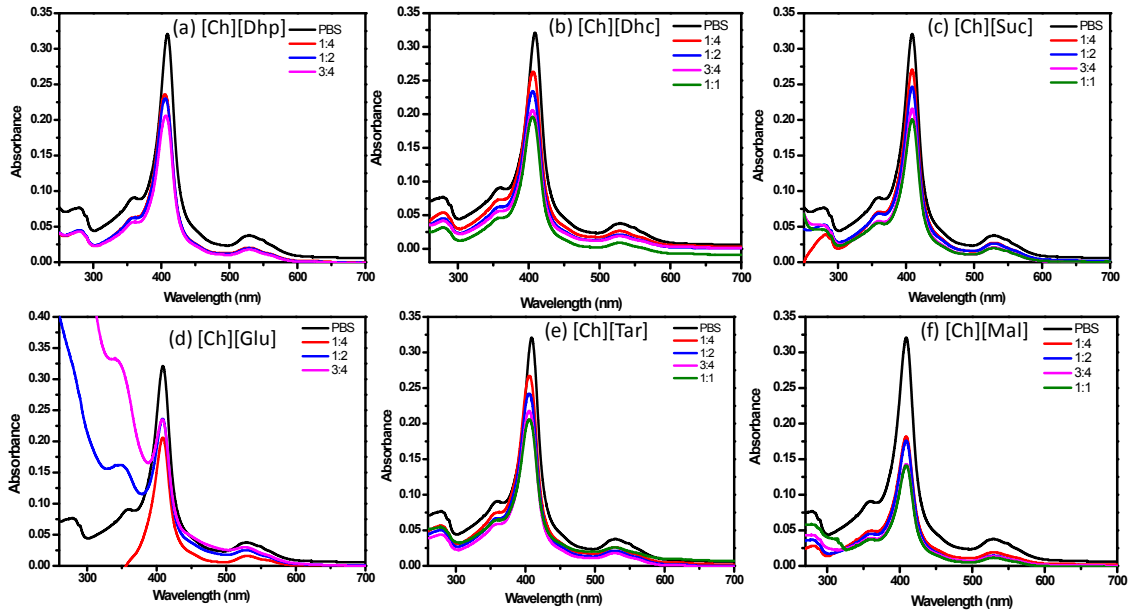

Fig. S2 UV-Vis spectra of Cyt C in aqueous solutions of bio-ILs at different concentration.

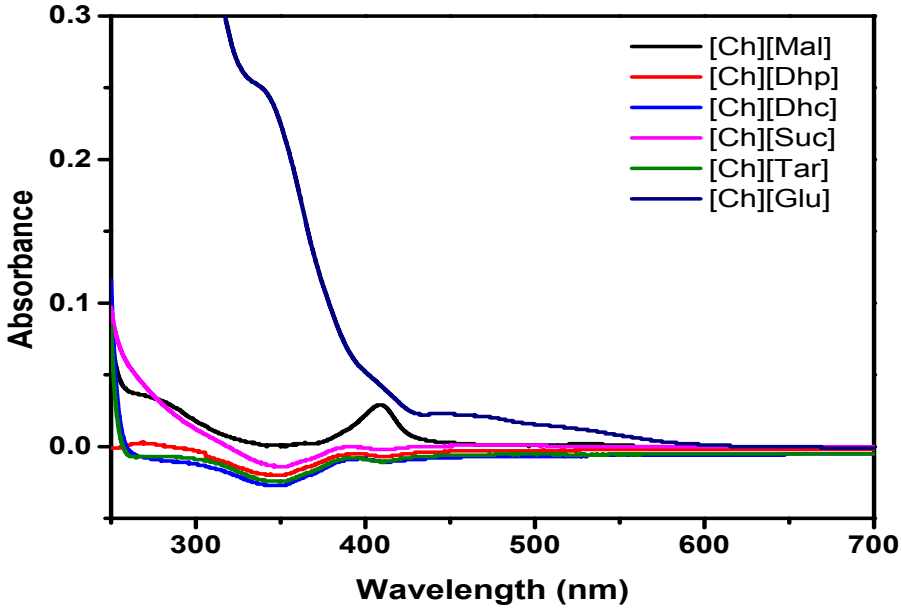

Fig. S3 UV-Vis spectra of aqueous solutions of ILs at 1:2 ratio by weight (IL:Water) (no enzyme added).

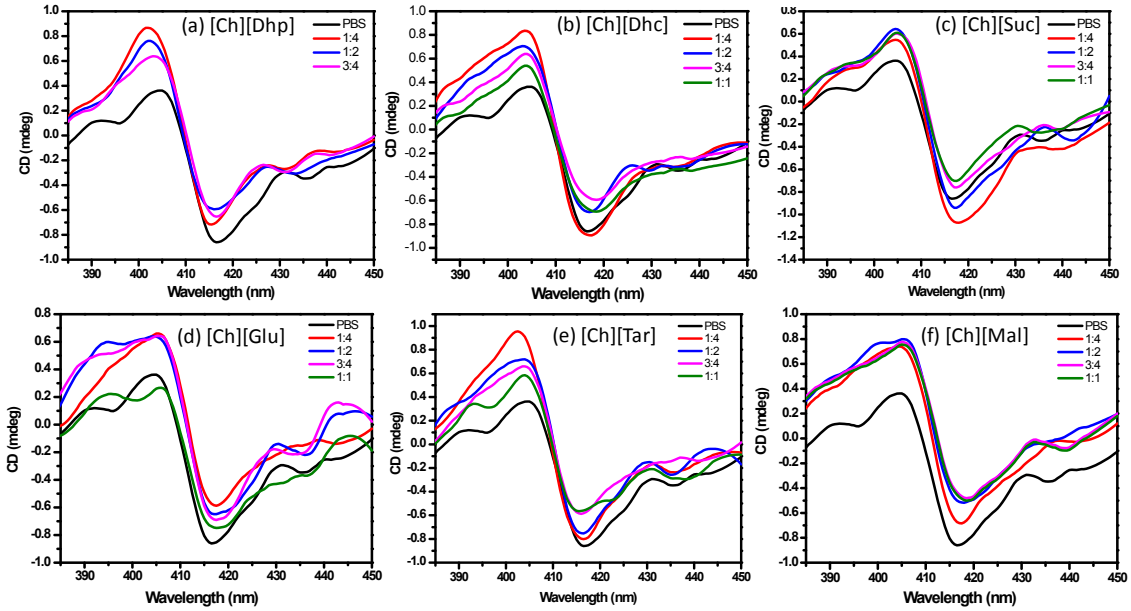

Fig. S4 CD spectra of Cyt C in aqueous solutions of cholinium-based ILs.

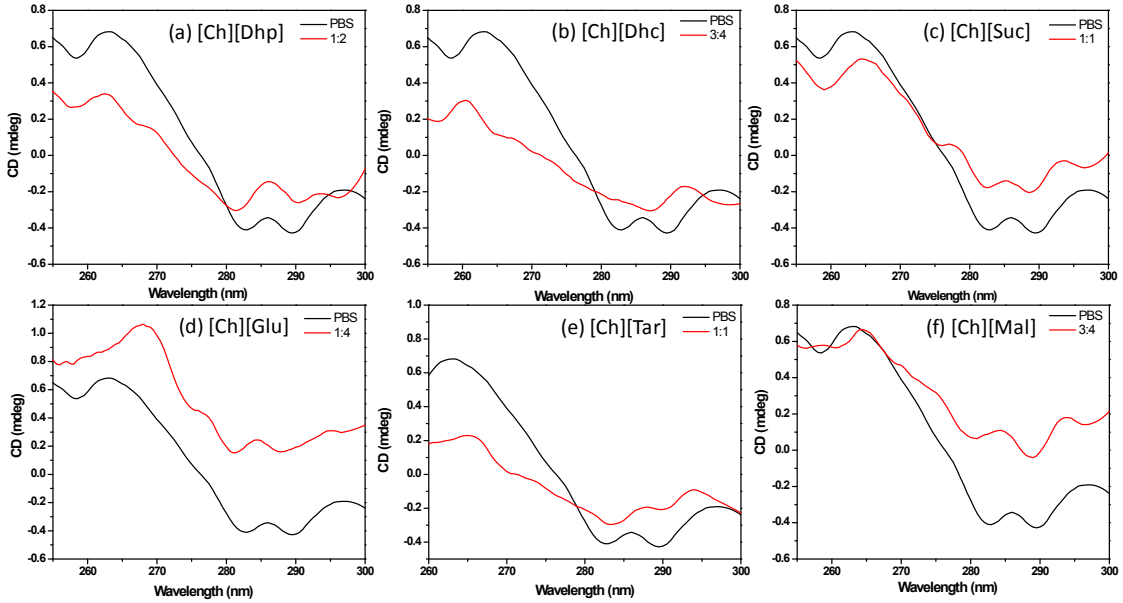

Fig. S5 Mid near UV CD spectra of Cyt C in presence of buffer and ILs.

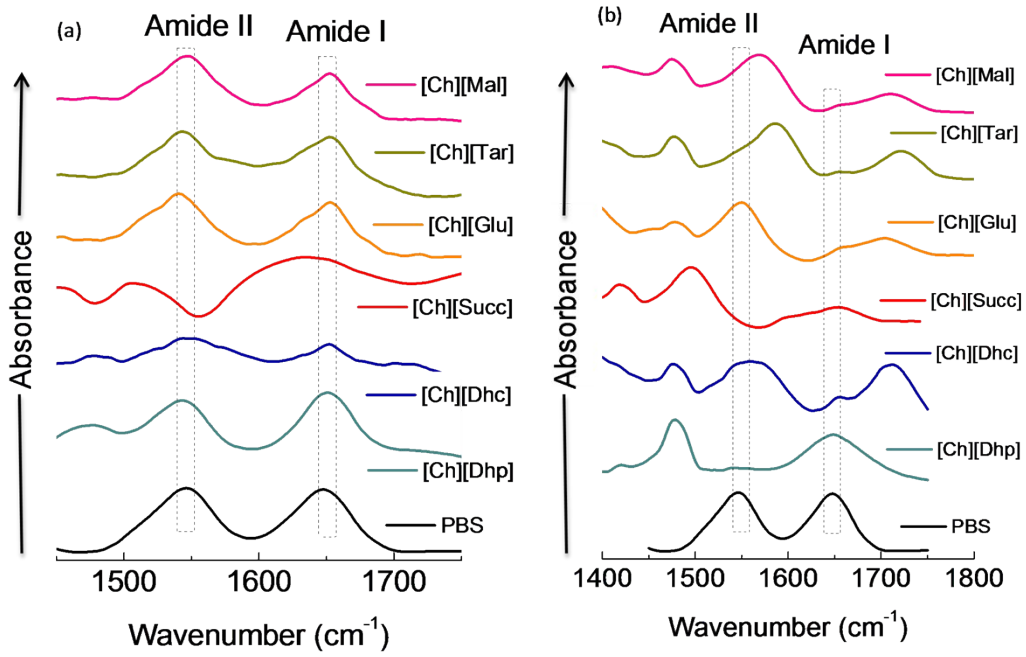

Fig. S6 FTIR spectra of Cyt C in the amide I and amide II regions at 25 °C: (a) Cyt C in aqueous solutions containing 1:2 weight ratio of ILs (b) Cyt C in aqueous solutions containing 3:4 of [Ch][Dhp] and 1:1 of the remaining ILs.

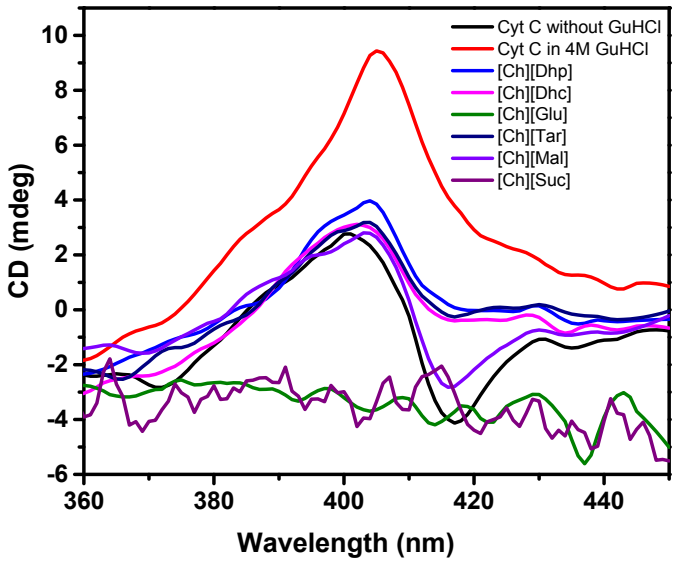

Fig. S7 CD spectra of Cyt C in presence of 4M GuHCl, with and without ILs added.

Table S1 T_{50} of enzyme in presence of different ILs.

Samples	T_{50} (°C)
PBS	101
[Ch][Dhp]	117
[Ch][Dhc]	110
[Ch][Suc]	114
[Ch][Glu]	115
[Ch][Tar]	114
[Ch][Mal]	113

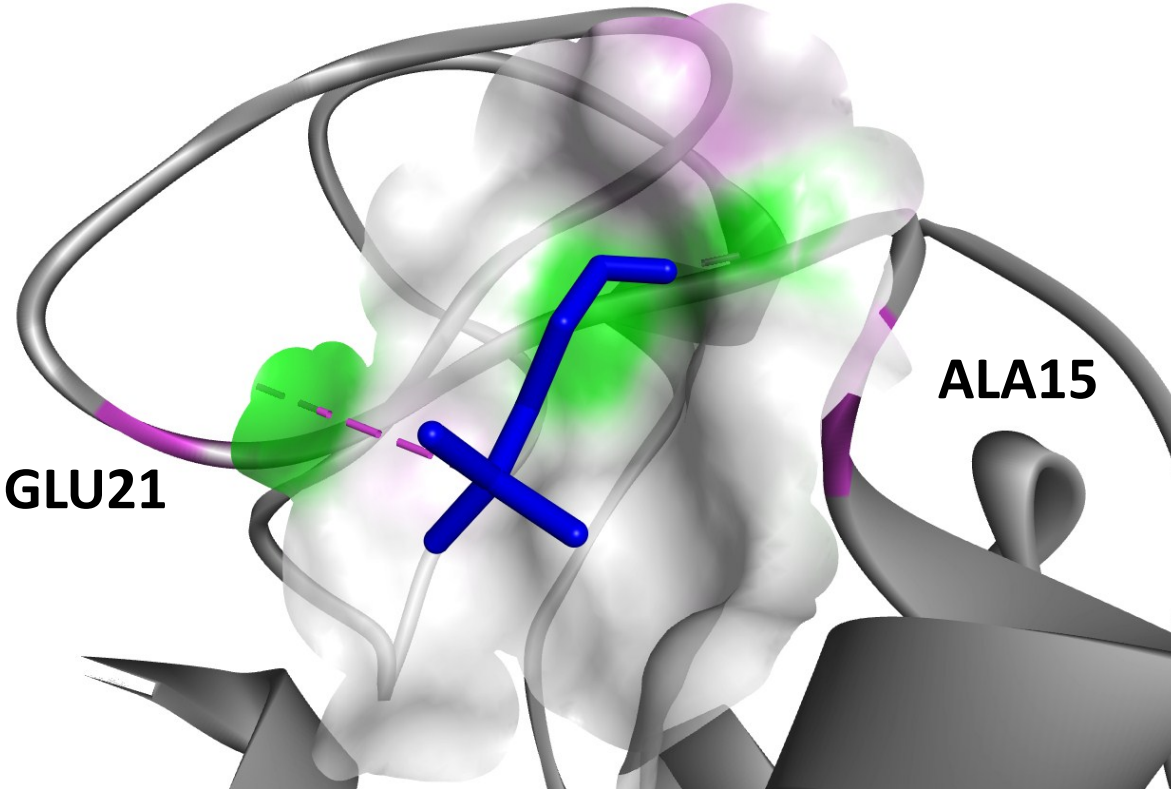

Figure S8 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Ch]^+$.

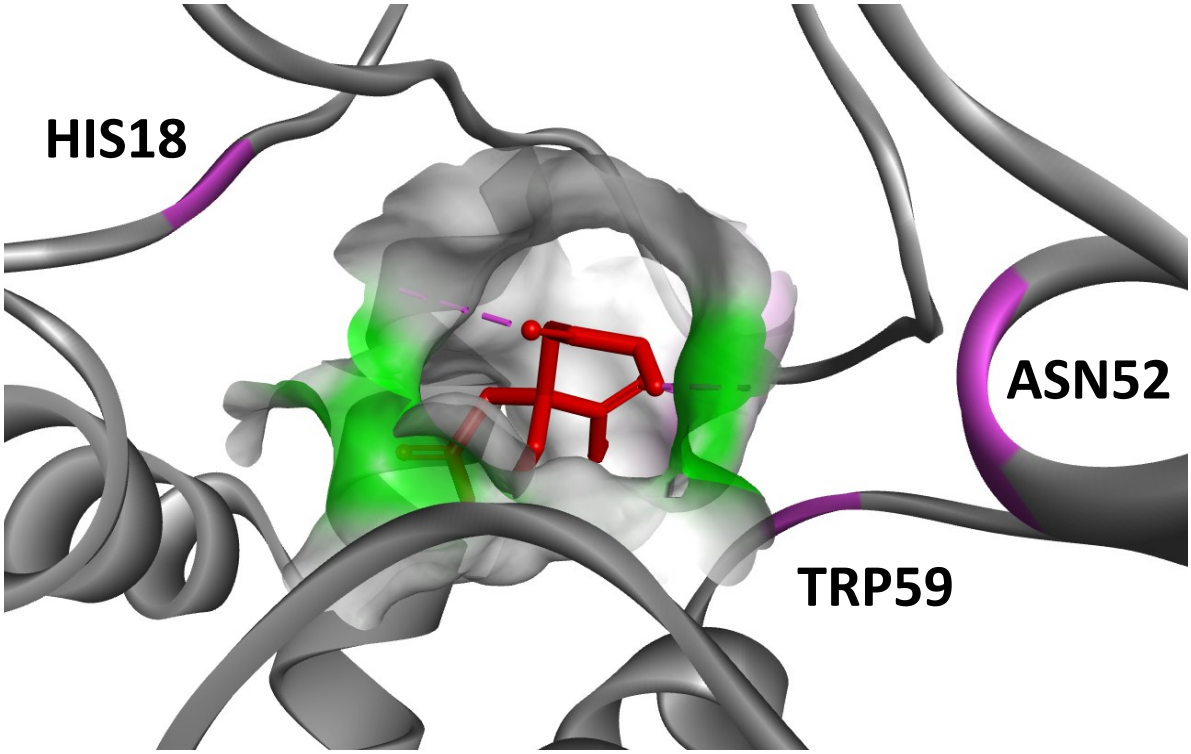

Figure S9 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Dhc]^-$.

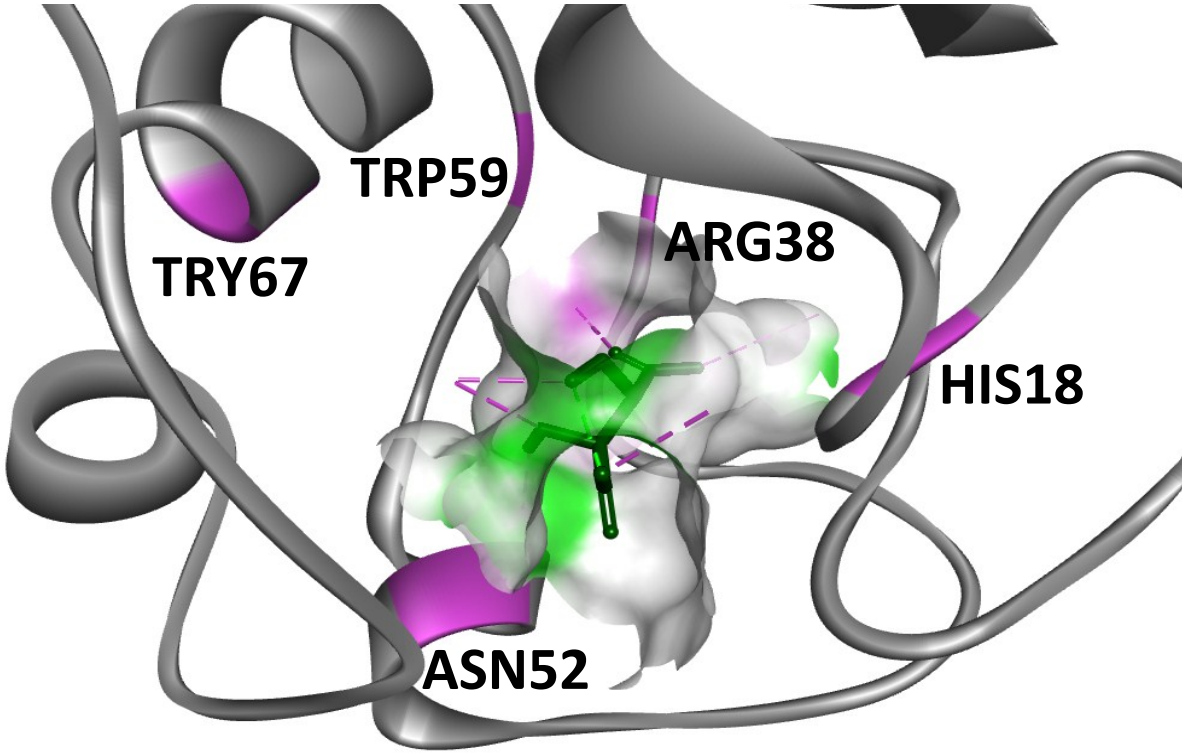

Figure S10 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Tar]^-$.

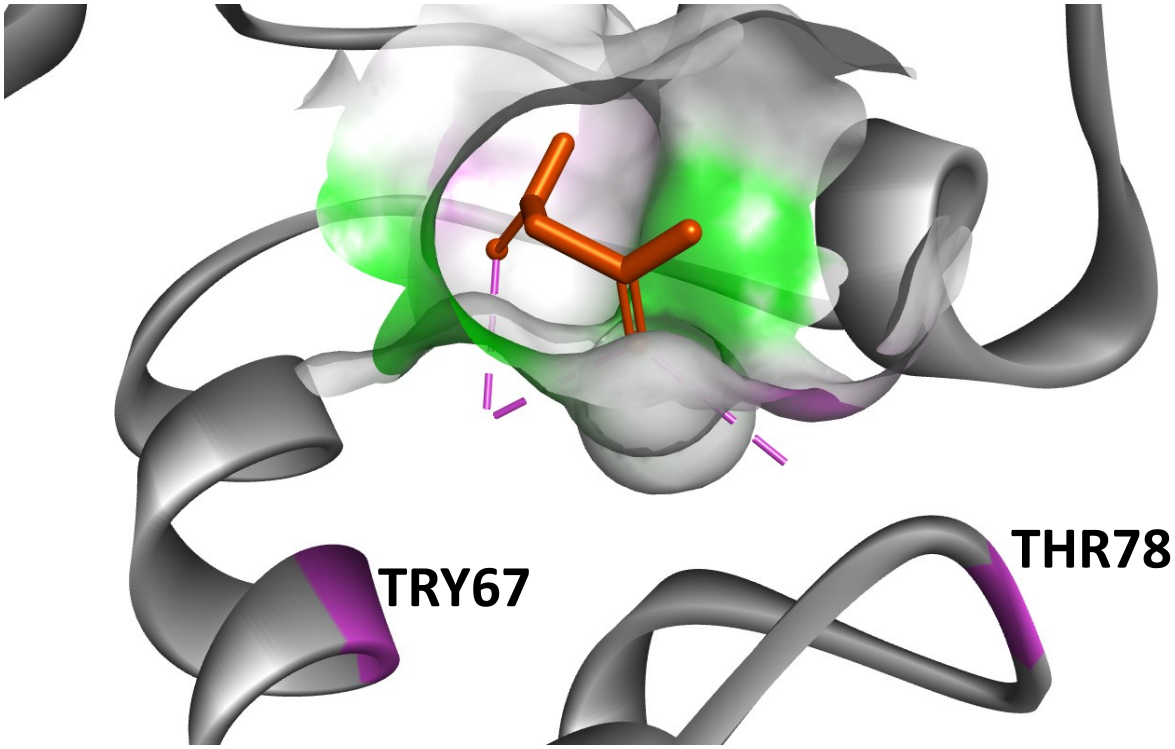

Figure S11 CytC docking pose with the lowest absolute value of affinity (kcal/mol) with $[Mal]^-$.

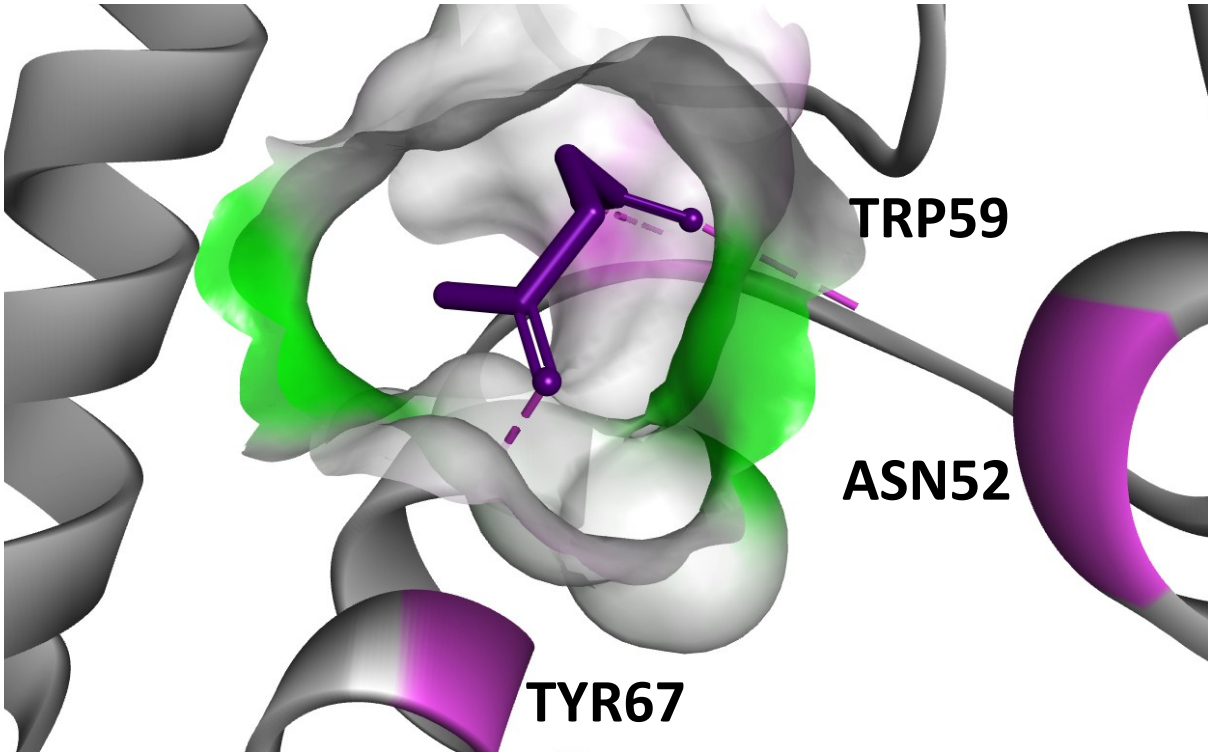

Figure S12 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Suc]^-$.

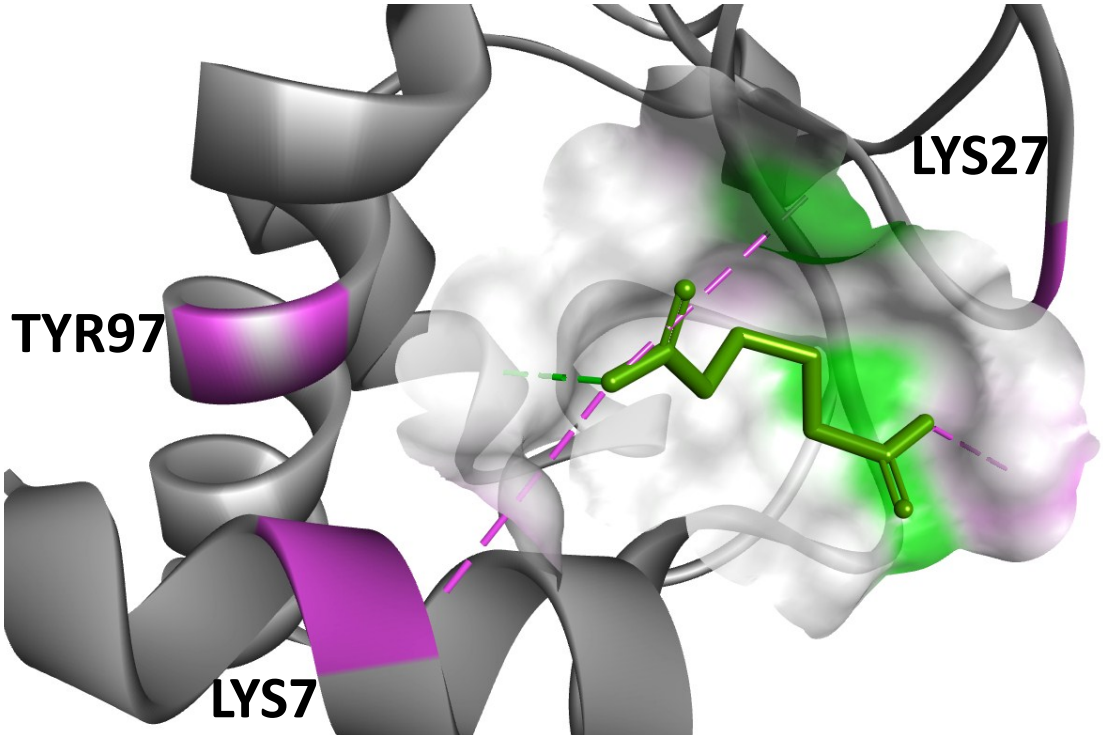

Figure S13 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Adi]^-$.

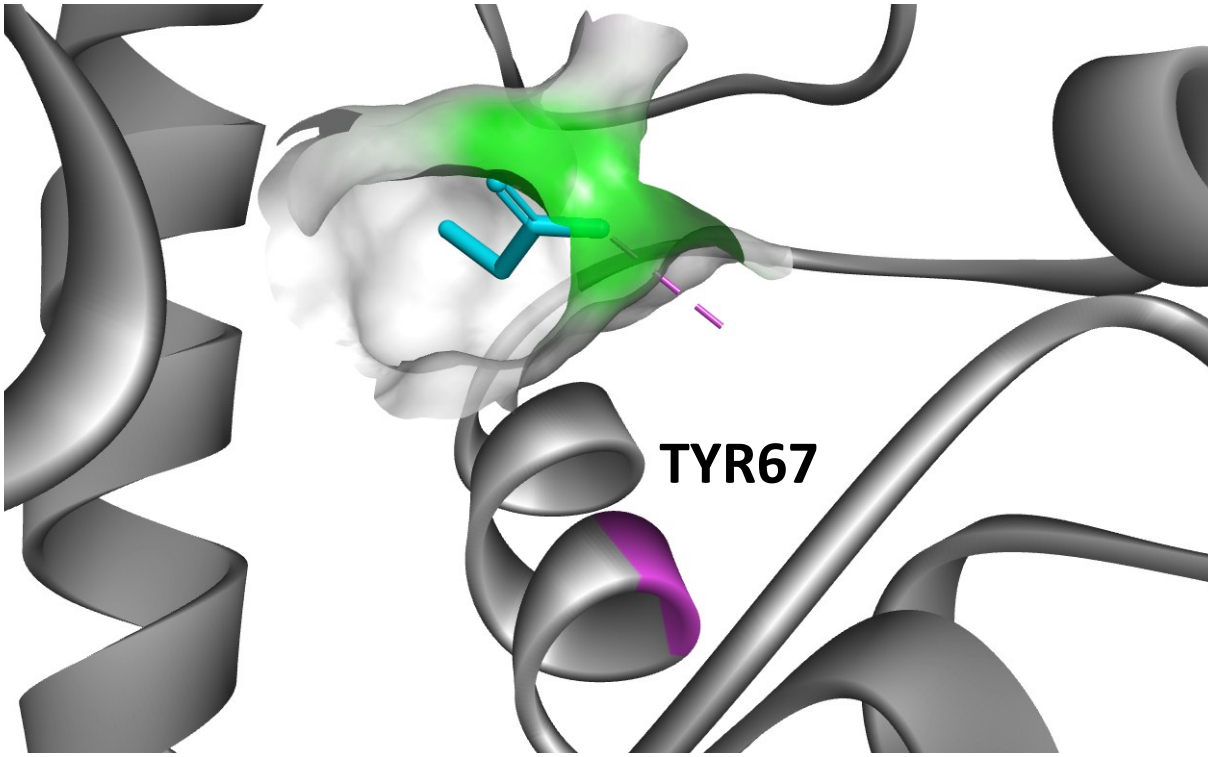

Figure S14 Cyt C docking pose with the lowest absolute value of affinity (kcal/mol) with $[Prop]^-$.

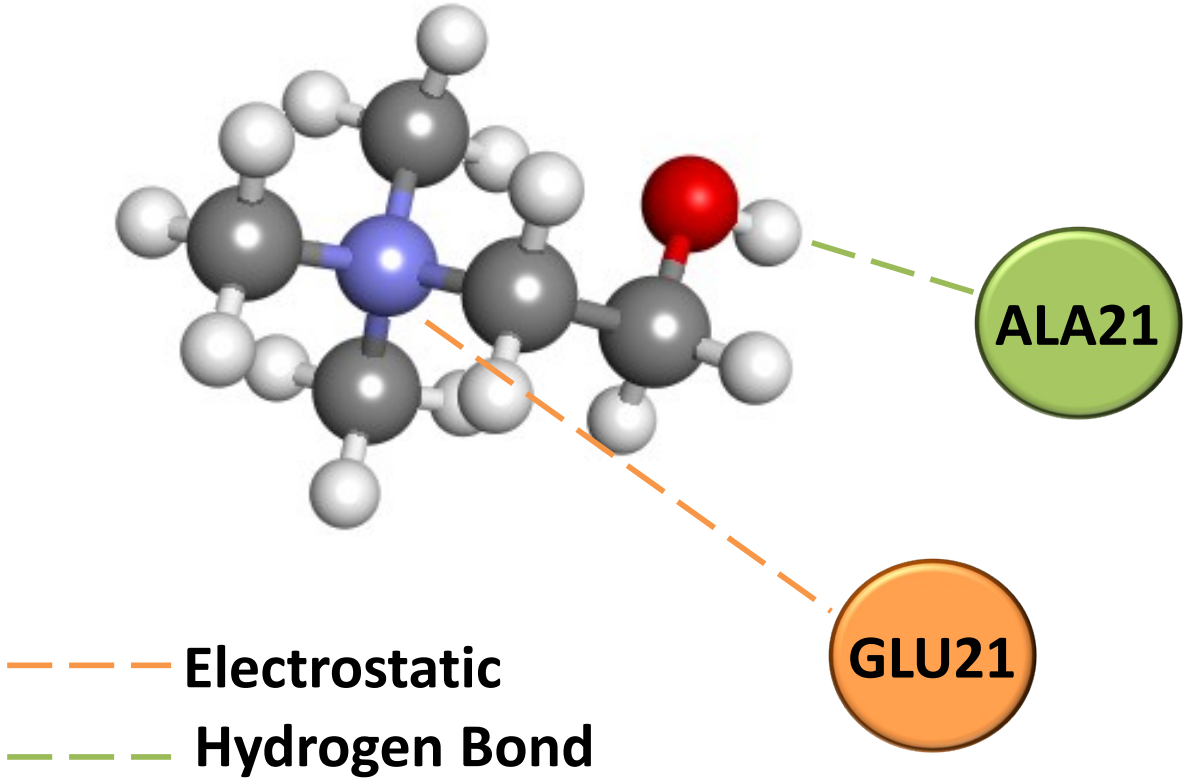

Figure S15 Molecular interactions representation between $[Ch]^+$ and the amino acids residues of Cyt C.

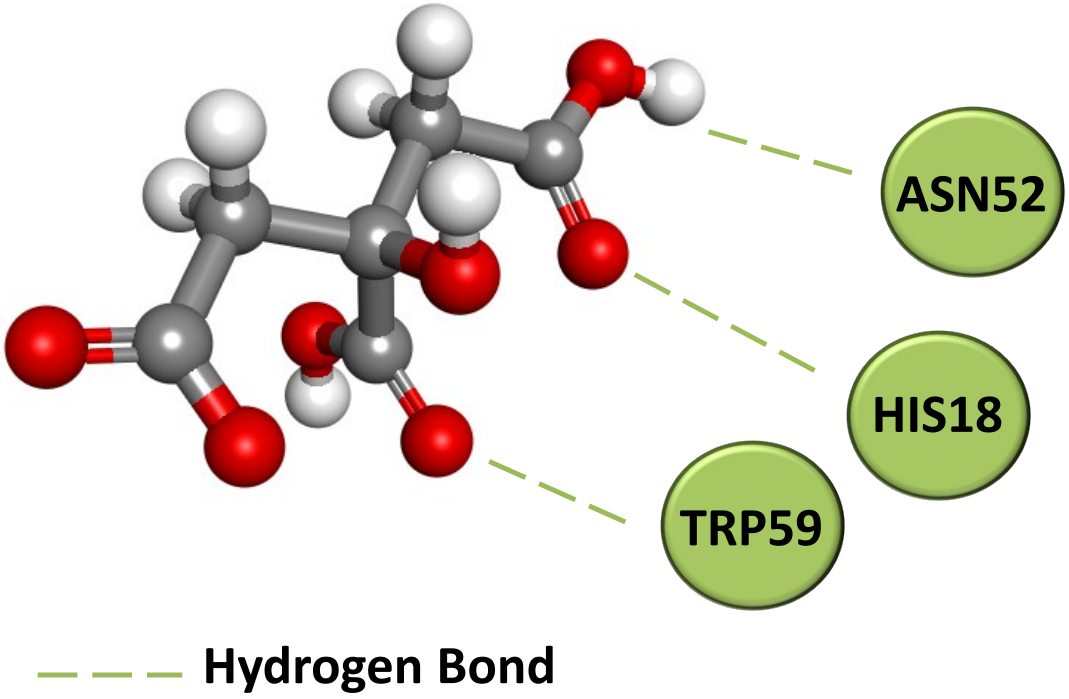

Figure S16 Molecular interactions representation of $[Dhc]^-$ and the amino acids residues of Cyt C.

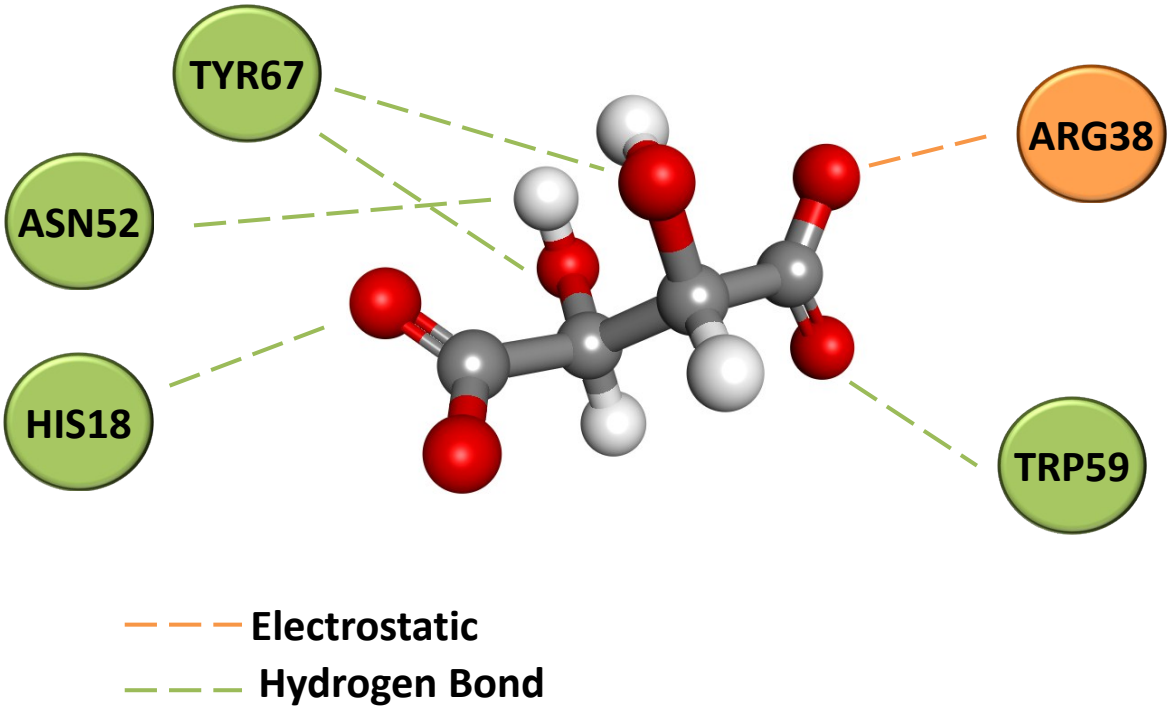

Figure S17 Molecular interactions representation of $[Tar]^-$ and the amino acids residues of Cyt C.

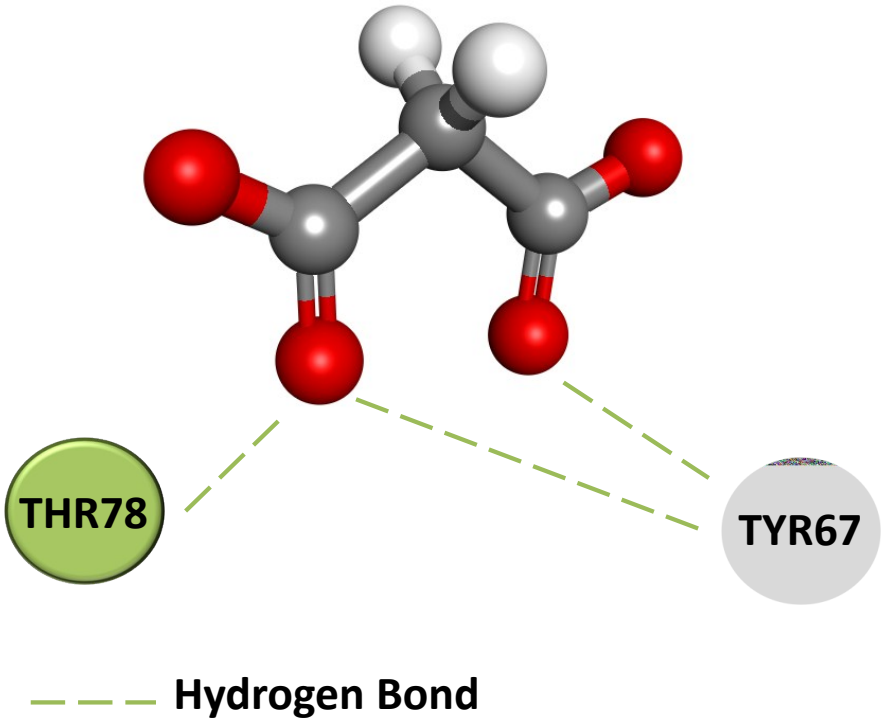

Figure S18 Molecular interactions representation of $[Mal]^-$ and the amino acids residues of Cyt C.

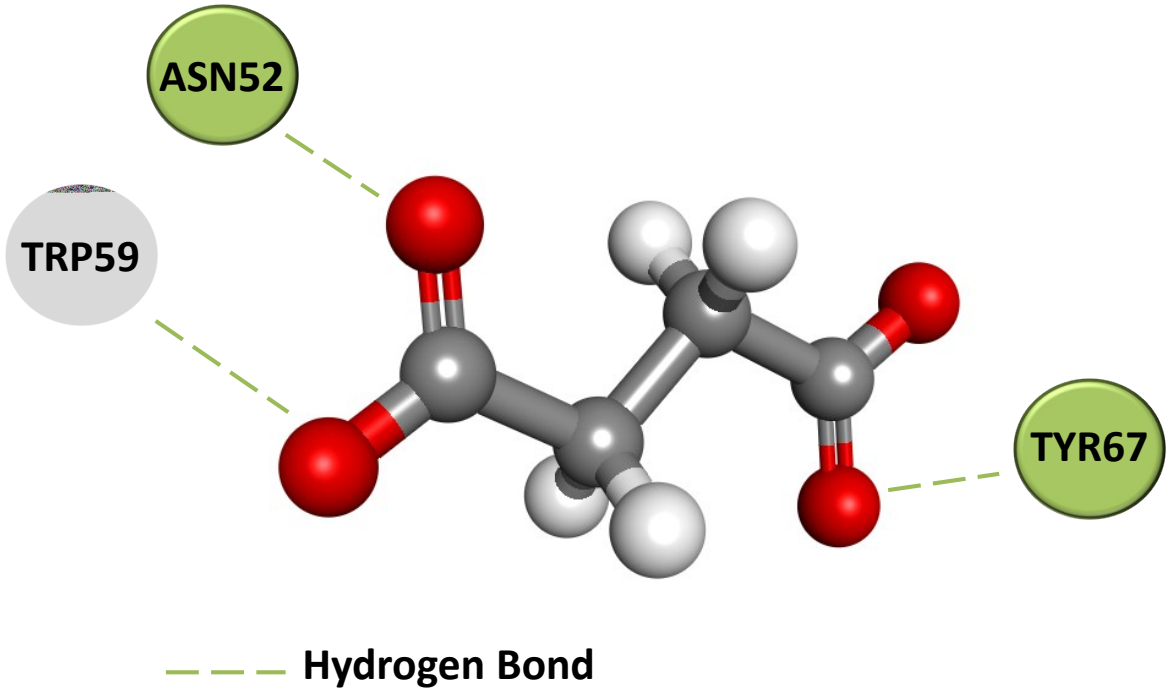

Figure S19 Molecular interactions representation of $[Suc]^-$ and the amino acids residues of Cyt C.

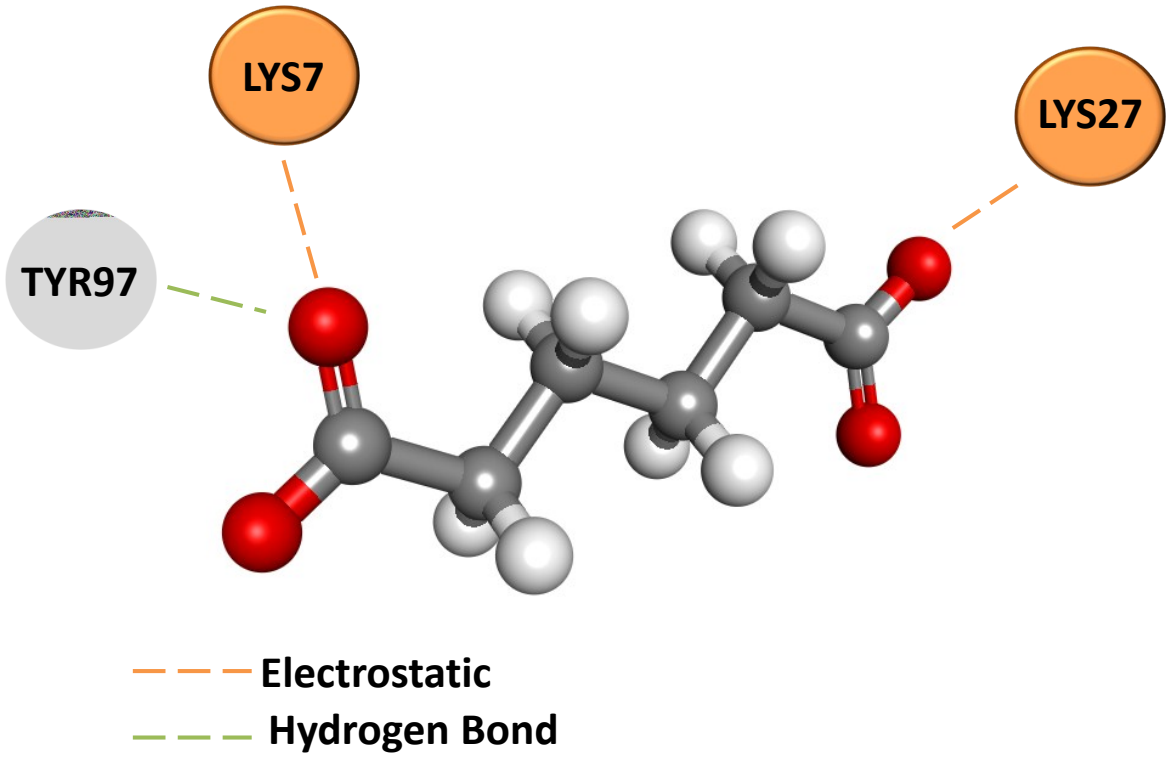

Figure S20 Molecular interactions representation of $[Adi]^-$ and the amino acids residues of Cyt C.

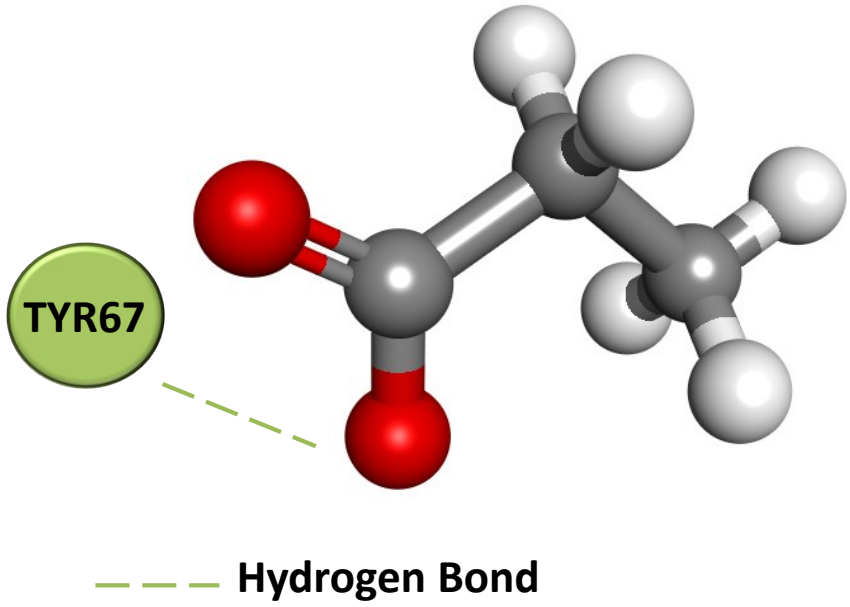

Figure S21 Molecular interactions represenation of $[Prop]^-$ and the amino acids residues of Cyt C.

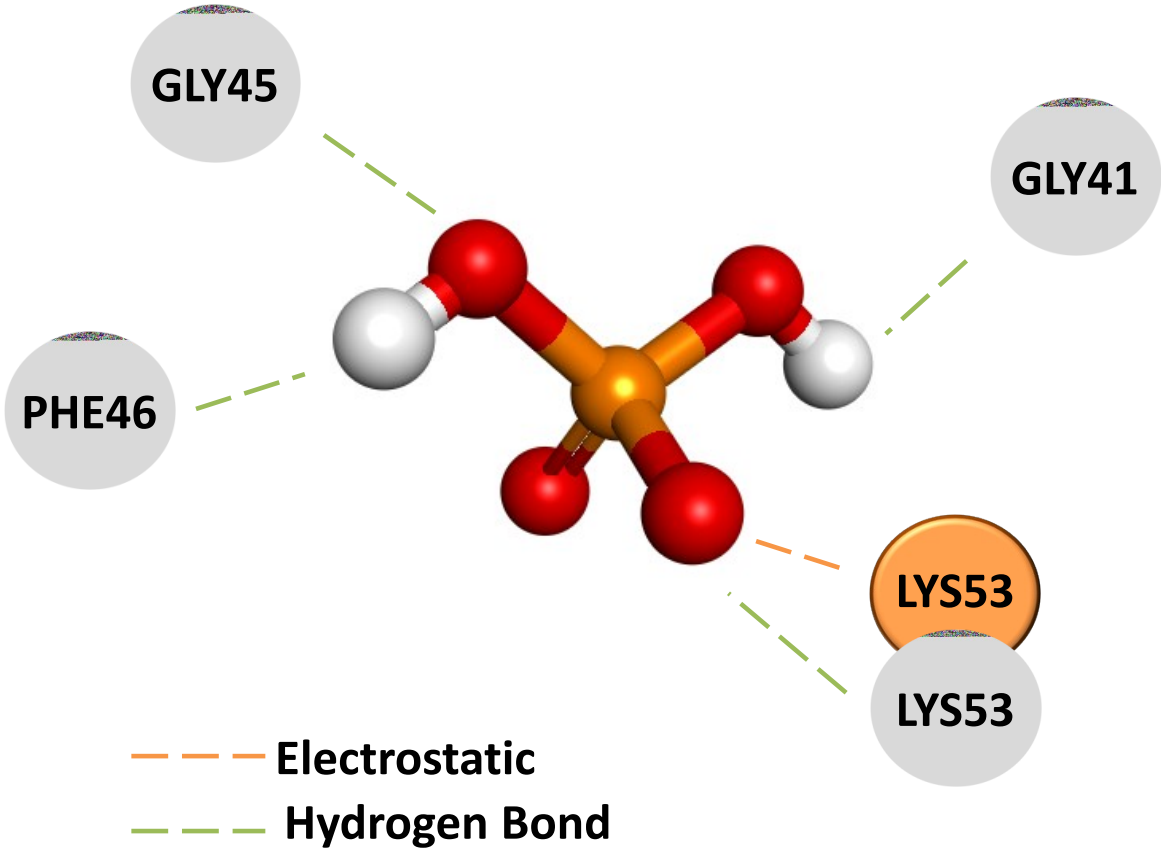

Figure S22 Molecular interactions representation of $[Dhp]^-$ and the amino acids residues of Cyt C.

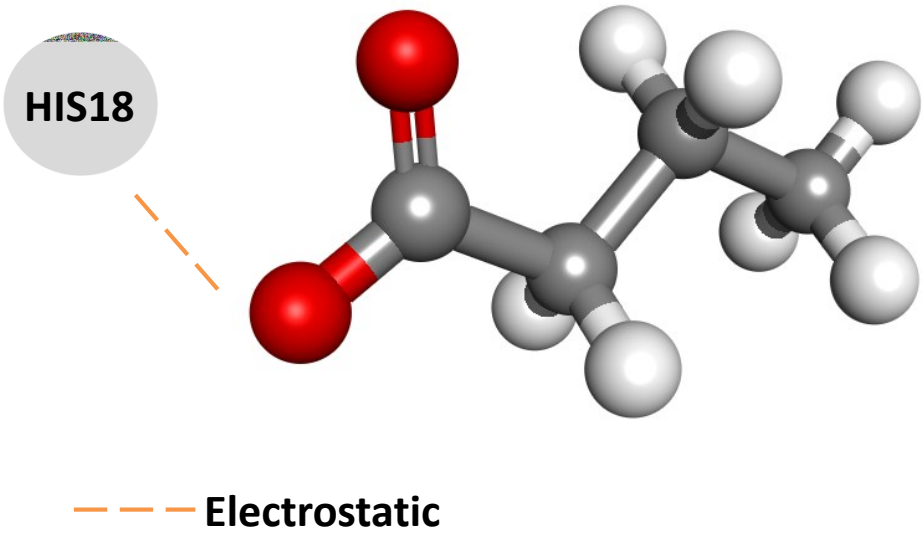

Figure S23 Molecular interactions representation of $[But]^-$ and the amino acids residues of Cyt C.

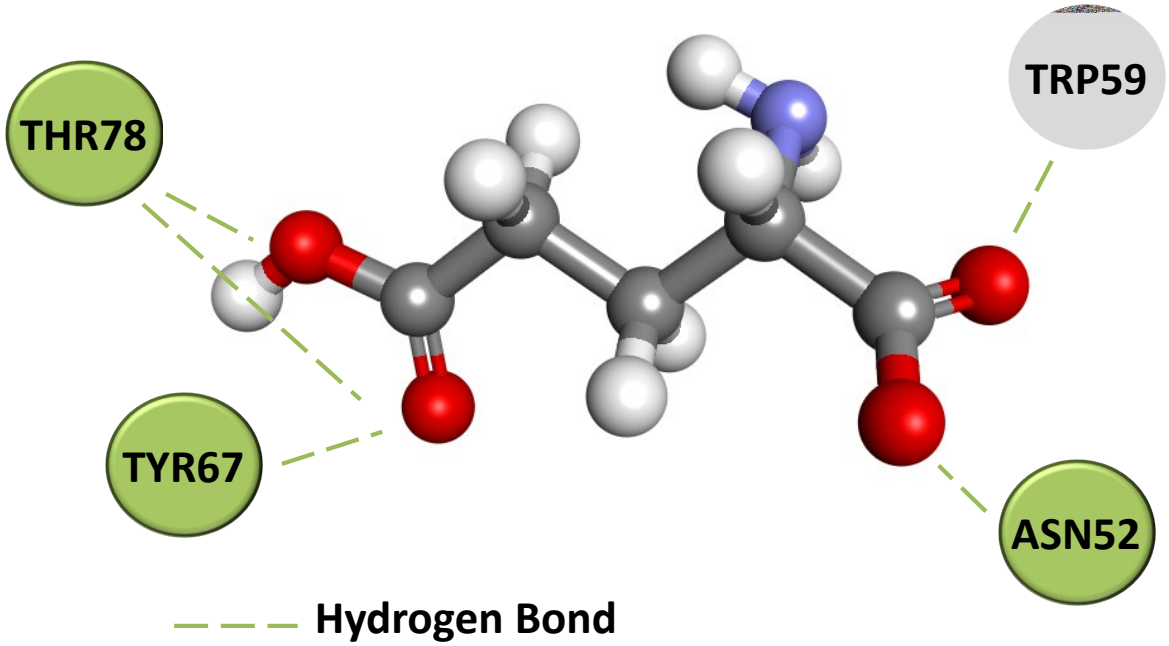

Figure S24 Molecular interactions representation of $[Glu]^-$ and the amino acids residues of Cyt C.

Table S2. Docking affinity energy and interacting amino acids predicted by AutoDock vinna for Cyt C-ILs.

IL ion	Affinity / (kcal/mol)	Interacting amino acids	Type of interaction	From	To	Distance / (Å)
$[Ch]^+$	-2.6	Glutamic acid21	Electostatic	$[Ch]^+$ - N	Glutamic acid - O	4.82
		Alanine15	Hydrogen Bond	$[Ch]^+$ - H	Alanine - O	1.92
$[Dhc]^-$	-4.4	Histidine18	Hydrogen Bond	Histidine - C	$[Dhc]^-$ - O	3.56
		Asparagine52		$[Dhc]^-$ - H	Asparagine- O	2.20
		Tryptophan59		Tryptophan - N	$[Dhc]^-$ - O	3.15
$[Dhp]^-$	-2.7	Glycine41	Hydrogen Bond	$[Dhp]^-$ - H	Glycine - H	2.75
		Glycine45		Glycine - N	$[Dhp]^-$ - O	3.08
		Phenylalanine46		$[Dhp]^-$ - H	Phenylalanine - O	2.21
		Lysine53		Lysine -C	$[Dhp]^-$ - O	3.55
			Electostatic	Lysine -N	$[Dhp]^-$ - O	3.96
$[Tar]^-$	-4.1	Arginine38	Electostatic	Arginine - H	$[Tar]^-$ - O	5.05
		Histidine18	Hydrogen Bond	Histidine - C	$[Tar]^-$ - O	3.56
		Asparagine52		$[Tar]^-$ - H	Asparagine - O	2.05
		Tryptophan59		Tryptophan - N	$[Tar]^-$ - O	3.18
		Tyrosine67		Tyrosine -OH	$[Tar]^-$ - O	2.98
				$[Tar]^-$ - H	Tyrosine -OH	2.74
$[Suc]^-$	-3.9	Asparagine52	Hydrogen Bond	Asparagine- N	$[Suc]^-$ - O	3.08
		Tryptophan59		Tryptophan - N	$[Suc]^-$ - O	2.98
		Tyrosine67		Tyrosine -OH	$[Suc]^-$ - O	2.94
$[Mal]^-$	3.3	Tyrosine67	Hydrogen Bond	Tyrosine -OH	$[Suc]^-$ - O	2.95

					[Suc]$^-$ - O	2.96
		Threonine78		Threonine -O	[Suc]$^-$ - O	3.22
IL ion	**Affinity / (kcal/mol)**	**Interacting amino acids**	**Type of interaction**	**From**	**To**	**Distance / (Å)**
[Glu]$^-$	-4.2	Asparagine52	Hydrogen Bond	Asparagine - N	[Glu]$^-$ - O	3.03
		Tryptophan59		Tryptophan - N	[Glu]$^-$ - O	3.03
		Tyrosine67		Tyrosine -OH	[Glu]$^-$ - O	2.91
		Threonine78		Threonine -O	[Glu]$^-$ - O	3.14
					[Glu]$^-$ - O	3.11
[Adi]$^-$	-3.4	Lysine27	Electostatic	Lysine -N	[Adi]$^-$- O	3.13
			Hydrogen Bond		[Adi]$^-$- O	5.21
		Tyrosine97		Tyrosine - OH	[Adi]$^-$- O	2.90
[But]$^-$	-3.2	Histidine18	Electostatic	[But]$^-$ - O	Histidine - C	3.36
[Prop]$^-$	-2.8	Tyrosine67	Hydrogen Bond	Tyrosine - OH	[Prop]$^-$- O	3.13