Supporting Information

A Computational Study of Anti-Cancer Drug Resistance caused by 10 Topisomerase I Mutations, Including 7 Camptothecin Analogs and Lucanthone

Kelly Mulholland and Chun Wu*

College of Science and Mathematics, Rowan University, Glassboro, NJ, 08028 USA

*To whom correspondence should be addressed: wuc@rowan.edu

Table **S1.** Decomposition of MM-GBSA binding energies (kcal/mol) for SN-38.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	4.0	8.4	-24.4	-89.2	-101.1	-	-	-	-	-
G363C	2.2	8.6	-15.9	-75.5	-80.5	-1.9	0.2	8.5	13.7	20.5
G717V	2.5	13.4	-16.3	-76.8	-77.3	-1.5	4.9	8.1	12.4	23.8
N722A	2.9	14.4	-15.9	-74.6	-73.2	-1.1	6.0	8.5	14.6	27.9
N722S	2.9	14.4	-15.9	-74.6	-73.2	-1.1	6.0	8.5	14.6	27.9
R364H	3.0	3.1	-16.7	-75.0	-85.6	-1.0	-5.3	7.7	14.2	15.5
G503S	2.4	16.5	-15.5	-80.4	-77.0	-1.6	8.1	8.8	8.8	24.1
D533N	2.9	14.9	-15.3	-77.7	-75.3	-1.2	6.5	9.1	11.4	25.8
F361S	2.3	5.7	-23.0	-55.2	-70.3	3.8	-2.8	1.4	34.0	30.8
T729A	6.9	6.8	-15.6	-75.4	-77.3	2.6	-1.7	8.8	13.8	23.7
D533G	14.7	24.3	-13.5	-61.9	-36.4	-4.0	15.8	10.9	27.3	64.7

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S2.** Decomposition of MM-GBSA binding energies (kcal/mol) for Topotecan.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	4.0	0.4	-25.8	-83.8	-105.2	-	-	-	-	-
G363C	3.6	3.7	-16.2	-81.0	-89.8	-0.4	3.3	9.6	2.8	15.4
G717V	3.8	4.1	-16.0	-83.2	-91.3	-0.3	3.7	9.8	0.6	13.9
N722A	4.2	10.6	-16.7	-78.9	-80.9	0.2	10.2	9.1	4.9	24.3
N722S	4.2	10.6	-16.7	-78.9	-80.9	0.2	10.2	9.1	4.9	24.3
R364H	4.3	-1.8	-17.0	-80.9	-95.4	0.3	-2.2	8.8	2.9	9.8
G503S	5.7	-1.5	-12.7	-76.9	-85.5	1.6	-1.9	13.1	6.9	19.7
D533N	3.1	18.8	-16.7	-83.6	-78.5	-1.0	18.4	9.1	0.2	26.7
F361S	5.0	-2.8	-12.8	-79.4	-89.9	0.9	-3.2	13.1	4.4	15.3
T729A	5.5	-0.7	-12.6	-78.5	-86.3	1.4	-1.1	13.2	5.3	18.9
D533G	4.7	1.0	-13.6	-77.1	-85.1	0.6	0.6	12.2	6.7	20.1

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S3.** Decomposition of MM-GBSA binding energies (kcal/mol) for Camptothecin.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	3.3	12.6	-22.8	-86.1	-93.1	-	-	-	-	-
G363C	1.3	13.6	-15.7	-76.7	-77.5	-2.0	1.0	7.1	9.4	15.5
G717V	1.0	16.6	-16.0	-77.7	-76.1	-2.3	4.0	6.8	8.4	17.0
N722A	2.0	18.7	-15.9	-76.0	-71.2	-1.3	6.1	7.0	10.1	21.8
N722S	2.0	18.7	-15.9	-76.0	-71.2	-1.3	6.1	7.0	10.1	21.8
R364H	1.9	6.2	-16.5	-75.6	-83.9	-1.4	-6.4	6.4	10.5	9.1
G503S	0.8	11.7	-15.2	-79.2	-81.9	-2.5	-0.9	7.6	6.9	11.1
D533N	1.3	14.5	-15.2	-77.0	-76.3	-2.0	1.9	7.7	9.2	16.7
F361S	6.8	15.8	-11.4	-65.3	-54.2	3.5	3.2	11.4	20.8	38.8
T729A	0.1	11.1	-15.3	-78.2	-82.3	-3.2	-1.5	7.5	7.9	10.7
D533G	6.9	10.7	-11.5	-69.9	-63.7	3.6	-1.9	11.4	16.2	29.3

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S4.** Decomposition of MM-GBSA binding energies (kcal/mol) for Exatecan.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	3.2	23.8	-33.0	-66.9	-72.9	-	-	-	-	-
G363C	6.0	38.9	-19.1	-65.1	-39.3	2.8	15.1	13.9	1.9	33.6
G717V	4.9	40.8	-19.4	-62.1	-35.8	1.7	17.0	13.6	4.9	37.1
N722A	7.3	44.6	-19.6	-64.2	-31.9	4.1	20.8	13.4	2.7	41.0
N722S	7.3	44.6	-19.6	-64.2	-31.9	4.1	20.8	13.4	2.7	41.0
R364H	10.6	42.0	-19.6	-52.6	-19.6	7.4	18.2	13.4	14.3	53.4
G503S	7.8	32.9	-18.7	-59.9	-38.0	4.6	9.1	15.7	7.0	35.0
D533N	3.0	39.7	-13.4	-62.2	-33.0	-0.2	15.9	21.1	4.7	40.0
F361S	5.7	15.9	-30.2	-45.5	-54.2	2.6	-7.9	12.1	21.4	18.8
T729A	4.2	35.9	-24.3	-71.7	-55.8	1.0	12.2	-1.7	-4.8	17.1
D533G	0.5	33.2	-22.2	-73.7	-62.2	-2.7	9.4	-1.8	-6.8	10.7

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S5.** Decomposition of MM-GBSA binding energies (kcal/mol) for Gimatecan.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	6.0	22.6	-34.3	-84.2	-89.8	-	-	-	-	-
G363C	6.9	36.1	-24.6	-75.8	-57.5	0.8	13.4	9.7	8.3	32.3
G717V	7.5	35.8	-25.0	-77.0	-58.7	1.4	13.2	9.3	7.2	31.1
N722A	6.0	36.4	-25.6	-74.6	-57.7	0.0	13.8	8.6	9.6	32.0
N722S	6.0	36.4	-25.6	-74.6	-57.7	0.0	13.8	8.6	9.6	32.0
R364H	5.8	24.4	-26.1	-75.7	-71.7	-0.3	1.7	8.2	8.5	18.1
G503S	2.3	34.2	-24.7	-79.8	-68.0	-3.7	11.6	9.6	4.4	21.8
D533N	6.0	40.6	-14.4	-71.6	-39.4	0.0	18.0	19.9	12.6	50.4
F361S	3.6	14.2	-33.0	-60.9	-76.0	-2.4	-8.4	1.3	23.3	13.8
T729A	7.5	36.7	-25.6	-74.8	-56.1	1.5	14.1	8.7	9.4	33.7
D533G	6.5	39.9	-14.9	-71.2	-39.7	0.4	17.2	19.4	13.0	50.1

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S6.** Decomposition of MM-GBSA binding energies (kcal/mol) for Belotecan.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	3.6	13.7	-32.6	-84.7	-100.0	-	-	-	-	-
G363C	12.7	25.1	-21.6	-70.4	-54.2	9.2	11.4	11.0	14.2	45.8
G717V	3.9	28.9	-23.6	-73.2	-64.0	0.3	15.2	9.0	11.5	36.0
N722A	-4.6	28.1	-21.5	-71.0	-69.0	-8.2	14.4	11.1	13.7	31.0
N722S	-4.6	28.1	-21.5	-71.0	-69.0	-8.2	14.4	11.1	13.7	31.0
R364H	4.8	13.0	-23.2	-66.6	-72.0	1.2	-0.7	9.4	18.1	28.0
G503S	9.3	22.4	-13.5	-68.5	-50.3	5.7	8.7	19.1	16.2	49.7
D533N	3.4	38.1	-23.6	-70.2	-52.2	-0.2	24.4	9.0	14.5	47.8
F361S	5.8	13.6	-30.3	-57.9	-68.7	2.2	0.0	2.3	26.8	31.3
T729A	8.9	26.0	-23.8	-66.7	-55.6	5.3	12.3	8.8	18.0	44.4
D533G	2.5	30.5	-21.7	-67.5	-56.2	-1.0	16.8	10.9	17.1	43.8

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S7.** Decomposition of MM-GBSA binding energies (kcal/mol) for Lucanthone.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	2.7	14.8	-24.3	-64.9	-71.7	-	-	-	-	-
G363C	9.1	20.1	-25.1	-56.9	-52.8	6.4	5.4	-0.8	8.0	18.9
G717V	3.3	15.9	-20.6	-60.0	-61.4	0.6	1.1	3.8	4.9	10.3
N722A	4.5	27.2	-15.5	-63.5	-47.3	1.7	12.4	8.8	1.4	24.4
N722S	4.5	27.2	-15.5	-63.5	-47.3	1.7	12.4	8.8	1.4	24.4
R364H	6.2	5.2	-18.9	-51.0	-58.5	3.4	-9.6	5.5	13.9	13.2
G503S	0.4	22.5	-17.4	-61.3	-55.9	-2.4	7.7	7.0	3.6	15.9
D533N	-0.3	25.8	-11.9	-61.5	-47.8	-3.0	11.1	12.5	3.4	24.0
F361	2.7	25.9	-13.2	-61.7	-46.3	0.0	11.1	11.2	3.2	25.5
T729A	2.2	19.2	-12.9	-65.0	-56.5	-0.5	4.4	11.4	-0.1	15.2
D533G	2.4	24.2	-14.7	-63.8	-51.9	-0.4	9.4	9.6	1.1	19.8

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S8.** Decomposition of MM-GBSA binding energies (kcal/mol) for Lurtotecan.

Target	Δ Conformation	Δ GBELE	Δ Hydrophobic	Δ VDW	Δ Sum	ΔΔ Conformation	ΔΔ GBELE	ΔΔ Hydrophobic	ΔΔ VDW	ΔΔ Sum
1K4T	13.0	-25.3	-21.1	-49.9	-83.4	-	-	-	-	-
G363C	16.0	-17.5	-21.0	-46.2	-68.8	3.0	7.8	0.1	3.7	14.6
G717V	23.8	-17.2	-21.5	-50.2	-65.1	10.9	8.1	-0.3	-0.3	18.3
N722A	26.4	-12.1	-20.5	-46.1	-52.3	13.4	13.2	0.6	3.9	31.1
N722S	26.4	-12.1	-20.5	-46.1	-52.3	13.4	13.2	0.6	3.9	31.1
R364H	39.4	-4.2	-22.5	-63.1	-50.4	26.4	21.0	-1.4	-13.1	32.9
G503S	26.0	-19.7	-21.5	-35.7	-50.8	13.1	5.6	-0.3	14.2	32.5
D533N	21.6	-17.3	-21.8	-37.5	-55.1	8.7	7.9	-0.7	12.4	28.3
F361S	26.5	-19.5	-21.5	-35.3	-49.8	13.6	5.7	-0.4	14.6	33.5
T729A	14.5	-13.1	-21.2	-51.4	-71.2	1.5	12.2	0.0	-1.4	12.2
D533G	20.6	-16.6	-22.3	-40.0	-58.3	7.6	8.7	-1.1	9.9	25.1

ΔG = ΔConformation + ΔGBELE + ΔHydrophobic + ΔVDW, GBELE= GB+Coulom+Hbond, Hydrophobic=LIPO, VDW= VDW+Pi stacking +self-contact correction. $\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wildtype}$

Table **S9.** Interacting residues on WT with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20	H20		H20		H20	77.8
		DT9				DT9		DT9	33.3
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
	DC111		DC111	DC111					33.3
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114		DA114	DA114	DA114	DA114	DA114	DA114	DA114	88.9
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351		ALA351	ALA351		ALA351	44.4
ASPN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
	LYS354				LYS354				22.2
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	100.0
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374		LYS374				22.2
					ILE377				11.1
			TRP416						11.1
			LYS425	LYS425	LYS425				33.3
			TYR426			TYR426		TYR426	33.3
			ILE427		ILE427	ILE427		ILE427	44.4
			MET428		MET428	MET428		MET428	44.4
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
	ARG488				ARG488			ARG488	33.3
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
					ASN631				11.1
	HID632				HID632			HID632	33.3
					GLN633				11.1
					ALA715				11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S10.** Interacting residues on D533G with ligands. The highlighted row indicates the mutated residue is present within 5Åof the ligand.

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
							DA13		11.1
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114	DA114	DA114	DA114	DA114	DA114		DA114	DA114	88.9
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
					ILE350				11.1
			ALA351		ALA351			ALA351	33.3
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374		LYS374				22.2
	TRP416		TRP416		TRP416			TRP416	44.4
	LYS425		LYS425		LYS425	LYS425		LYS425	55.6
	TYR426		TYR426		TYR426	TYR426		TYR426	55.6
			ILE427		ILE427			ILE427	33.3
			MET428		MET428			MET428	33.3
			LEU429		LEU429				22.2
			ASN430						11.1
			PRO431						11.1
			LYS436		LYS436				22.2
ARG488	ARG488	ARG488	ARG488	ARG488	ARG488	ARG488		ARG488	88.9
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
GLY533	GLY533	GLY533	GLY533	GLY533	GLY533	GLY533	GLY533	GLY533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	100.0
	HIS632								11.1
THR718	THR718	THR718		THR718	THR718	THR718	THR718	THR718	88.9
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S11.** Interacting residues on D533N with ligands. The highlighted row indicates the mutated residue is present within 5Åof the ligand.

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
							DA13		11.1
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114			DA114	DA114	DA114		DA114	DA114	66.7
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
					ILE350				11.1
			ALA351		ALA351			ALA351	33.3
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
				LYS354					11.1
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374						11.1
	TRP416		TRP416		TRP416			TRP416	44.4
	LYS425		LYS425	LYS425	LYS425	LYS425		LYS425	66.7
	TYR426		TYR426	TYR426	TYR426	TYR426		TYR426	66.7
					ILE427			ILE427	22.2
			MET428		MET428			MET428	33.3
			LEU429		LEU429				22.2
			ASN430						11.1
			PRO431						11.1
			LYS436		LYS436				22.2
ARG488	ARG488	ARG488				ARG488		ARG488	55.6
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASN533	ASN533	ASN533	ASN533	ASN533	ASN533	ASN533	ASN533	ASN533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
						HIS632			11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
				LEU721			LEU721		22.2
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S12.** Interacting residues on F361S with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20			H20		H20	66.7
	DT9	DT9	DT9					DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
					DA13				11.1
					DA14				11.1
					DA15				11.1
					DT110				11.1
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114		DA114	DA114	DA114	DA114		DA114	DA114	77.8
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
					MET263				11.1
					HIS266				11.1
					TYR268				11.1
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352		ASN352	ASN352	ASN352	88.9
				LYS354					11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
					GLY363				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374						11.1
			TRP416					TRP416	22.2
	LYS425		LYS425	LYS425		LYS425		LYS425	55.6
	TYR426			TYR426		TYR426		TYR426	44.4
								ILE427	11.1
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
ARG488	ARG488	ARG488			ARG488	ARG488	ARG488	ARG488	88.9
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535	ILE535	ILE535	ILE535	ILE535	ILE535	100.0
					HIS632		HIS632		22.2
					GLN633		GLN633		22.2
					ARG634				11.1
					ALA635				11.1
							ALA715		11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S13.** Interacting residues on G363C with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9			DT9				22.2
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
			DC111						11.1
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114	DA114	DA114	DA114	DA114	DA114				66.7
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
				LYS354					11.1
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
					PHE361				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
					LYS374				11.1
					ARG375				11.1
					ILE377				11.1
	TRP416		TRP416		TRP416				33.3
					THR417				11.1
					GLU418				11.1
					ILE420				11.1
	LYS425		LYS425	LYS425	LYS425	LYS425		LYS425	66.7
	TYR426		TYR426	TYR426	TYR426			TYR426	55.6
			ILE427					ILE427	22.2
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
ARG488	ARG488	ARG488			ARG488	ARG488	ARG488	ARG488	77.8
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
							ASN631		11.1
	HIS632						HIS632	HIS632	33.3
							GLN633		11.1
							ARG634		11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S14.** Interacting residues on G503S with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20			H20		H20	66.7
			DT9					DT9	22.2
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
					DA13				11.1
					DA14				11.1
					DA15				11.1
	DC111								11.1
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114		DA114	DA114	DA114	DA114			DA114	66.7
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
					TYR268				11.1
					HIS266				11.1
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352		ASN352	ASN352	ASN352	88.9
	LYS354			LYS354					22.2
GLU356	GLU356	GLU356	GLU356	GLU356		GLU356		GLU356	77.8
					ARG362				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374						11.1
								TRP416	11.1
	LYS425		LYS425	LYS425		LYS425		LYS425	55.6
				TYR426		TYR426		TYR426	33.3
								ILE427	11.1
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
ARG488	ARG488	ARG488			ARG488	ARG488	ARG488	ARG488	88.9
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	100.0
					HIS632		HIS632		22.2
					GLN633		GLN633		22.2
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
				LEU721					11.1
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S15.** Interacting residues on G717V with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
			DC111	DC111					22.2
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114	DA114	DA114	DA114	DA114	DA114			DA114	77.8
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
					LYS354				11.1
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
					PRO357				11.1
					PRO358				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
					GLY369				11.1
			LYS374		LYS374				22.2
					ARG375				11.1
	TRP416		TRP416		TRP416				33.3
					GLU418				11.1
					ASN419				11.1
					ILE420				11.1
	LYS425		LYS425	LYS425	LYS425	LYS425		LYS425	66.7
	THR426		TYR426	TYR426	TYR426		TYR426	TYR426	66.7
			ILE427					ILE427	22.2
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
ARG488	ARG488	ARG488			ARG488	ARG488		ARG488	66.7
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
							LEU721		11.1
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S16.** Interacting residues on N722A with ligands. The highlighted row indicates the mutated residue is present within 5Åof the ligand.

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12		DG12	88.9
	DC111	0	DC111						22.2
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114		DA114	DA114	DA114	DA114			DA114	66.7
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
							PHE353		11.1
	LYS354	0		LYS354			LYS354		33.3
							ILE3355		11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	100.0
					PRO357				11.1
					PRO358				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374		LYS374				22.2
					ARG375				11.1
					ILE377				11.1
			TRP416		TRP416		TRP416		33.3
					THR417				11.1
					GLU418				11.1
					ASN419				11.1
					ILE420				11.1
	LYS425		LYS425	LYS425	LYS425	LYS425		LYS425	66.7
			TYR426	TYR426	TYR426	TYR426	TYR426	TYR426	66.7
			ILE427				ILE427	ILE427	33.3
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
ARG488	ARG488	ARG488			ARG488	ARG488		ARG488	66.7
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532		LYS532	88.9
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533		ASP533	88.9
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535		ILE535	77.8
								HIS632	11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718		THR718	88.9
				LEU721					11.1
ALA722	ALA722	ALA722	ALA722	ALA722	ALA722	ALA722	ALA722	ALA722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723		PTR723	88.9

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S17.** Interacting residues on N722S with ligands. The highlighted row indicates the mutated residue is present within 5Åof the ligand.

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12		DG12	88.9
	DC111		DC111						22.2
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DA114		DA114	DA114	DA114	DA114			DA114	66.7
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351					ALA351	22.2
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
							PHE353		11.1
	LYS354			LYS354			LYS354		33.3
							ILE3355		11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	100.0
					PRO357				11.1
					PRO358				11.1
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374		LYS374				22.2
					ARG375				11.1
					ILE377				11.1
			TRP416		TRP416		TRP416		33.3
					THR417				11.1
					GLU418				11.1
					ASN419				11.1
					ILE420				11.1
	LYS425		LYS425	LYS425	LYS425	LYS425		LYS425	66.7
			TYR426	TYR426	TYR426	TYR426	TYR426	TYR426	66.7
			ILE427				ILE427	ILE427	33.3
			MET428					MET428	22.2
			LEU429						11.1
			ASN430						11.1
			PRO431						11.1
			LYS436						11.1
ARG488	ARG488	ARG488			ARG488	ARG488		ARG488	66.7
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532		LYS532	88.9
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533		ASP533	88.9
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535		ILE535	77.8
								HIS632	11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718		THR718	88.9
				LEU721					11.1
SER722	SER722	SER722	SER722	SER722	SER722	SER722	SER722	SER722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723		PTR723	88.9

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S18**. Interacting residues on R364H with ligands. The highlighted row indicates the mutated residue is present within 5Åof the ligand.

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20		H20	H20		H20	77.8
		DT9	DT9		DT9			DT9	44.4
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	DG12	100.0
	DC111			DC111	DC111				33.3
DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
		DA114						DA114	22.2
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
			ALA351		ALA351			ALA351	33.3
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
	LYS354				LYS354				22.2
	ILE355				ILE355				22.2
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
HIS364	HIS364	HIS364	HIS364	HIS364	HIS364	HIS364	HIS364	HIS364	100.0
			LYS374						11.1
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
					TRP416				11.1
	LYS425		LYS425	LYS425		LYS425		LYS425	55.6
				TYR426	TYR426	TYR426		TYR426	44.4
					ILE427			ILE427	22.2
			MET428		MET428			MET428	33.3
			LEU429		LEU429				22.2
			ASN430						11.1
			PRO431						11.1
			LYS436		LYS436				22.2
ARG488	ARG488	ARG488	ARG488		ARG488	ARG488	ARG488	ARG488	88.9
							ASN631		11.1
						HIS632	HIS632	HIS632	33.3
							GLN633		11.1
							ALA715		11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
				LEU721			LEU721		22.2
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S19.** Interacting residues on T729A with ligands

Camptothecin	Topotecan	LE-SN38	Lurotecan	Exatecan	Irinotecan	Belotecan	Luanthone	Gimatecan	%
H20	H20	H20	H20	H20	H20	H20		H20	88.9
DG12	DG112		DG12	DG12	DG12	DG12	DG12	DG12	88.9
DA114		DA114	DA114	DA114	DA114				55.6
DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	DT10	100.0
DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	DA113	100.0
DC112	DA112	DC112	DC112	DC112	DC112	DC112	DC112	DC112	100.0
			DT9		DT9			DT9	33.3
							DA13		11.1
					DC8				11.1
TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	TGP11	100.0
	ALA351		ALA351		ALA351	ALA351		ALA351	55.6
ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	100.0
					ILE355				11.1
GLU356	GLU356	GLU356	GLU356	GLU356	GLU356	GLU356		GLU356	88.9
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	100.0
			LYS374						11.1
			TRP416		TRP416				22.2
	LYS425		LYS425	LYS425	LYS425			LYS425	55.6
	TYR426		TYR426	TYR426	TYR426	TYR426		TYR426	66.7
	ILE427		ILE427		ILE427	ILE427		ILE427	55.6
			MET428		MET428	MET428		MET428	44.4
			LEU429		LEU429				22.2
			ASN430		ASN430				22.2
			PRO431						11.1
			LYS436		LYS436				22.2
ARG488	ARG488	ARG488	ARG488	ARG488	ARG488	ARG488		ARG488	88.9
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	100.0
ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	100.0
ILE535	ILE535	ILE535	ILE535		ILE535	ILE535	ILE535	ILE535	88.9
							GLN633		11.1
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	100.0
ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	ASN722	100.0
PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	PTR723	100.0

*The distance cutoff between a ligand and contacting residues/water molecules is 5Å.

Table **S20.** RMSD(Å) for each ligand-mutant complex

	Topotecan	Camptothecin	Lucanthone	Lurtotecan	LESN38	Gimatecan	Exatecan	Belotecan
WT	0.158	0.422	0.482	0.301	0.399	1.057	0.153	0.233
D533G	0.169	0.224	0.700	0.415	0.446	0.231	0.125	0.216
D533N	0.191	0.331	0.362	0.628	0.157	0.155	0.218	0.716
N722A	0.339	0.368	0.321	0.273	0.168	0.353	0.122	0.644
N722S	0.339	0.368	0.321	0.273	0.168	0.353	0.122	0.644
G363C	0.131	0.358	0.144	0.287	0.164	1.044	0.128	0.518
G503S	0.301	0.237	0.429	0.588	0.205	0.140	0.144	0.272
F361S	0.567	0.297	0.969	0.485	0.496	0.302	1.444	0.153
G717V	0.131	0.358	0.144	0.287	0.164	1.044	0.128	0.518
R364H	0.370	0.348	0.207	0.865	0.117	0.546	0.215	0.232
T729A	0.128	0.349	0.169	0.533	0.400	1.040	0.382	0.604

Table **S21.** Complexes bound to topotecan chosen for MD system

Complex	ΔG (kcal/mol)	Selected for MD
WT	-105.21	X
R364H	-95.44	X
G717V	-91.31	
F361S	-89.94	X
G363C	-89.85	
T729A	-86.33	X
G503S	-85.46	X
D533G	-85.08	X
N722A	-80.91	
N722S	-80.91	X

Table **S22.** Interacting residues (5Å) on topotecan bound to wild type and mutant complex using both docking and MD simulations

WT		D533G		F361S		G503S		N722S		R364H		T729A	
MD	**Dock**	**MD**	**Dock**	**MD**	**Dock**	**MD**	**Dock**	**MD**	**Dock**	**MD**	**Dock**	**MD**	**Dock**
ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364	ARG364			ARG364	ARG364
LYS425		LYS425	LYS425		LYS425	LYS425	LYS425	LYS425	LYS425	LYS425	LYS425	LYS425	LYS425
LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532	LYS532
ASP533	ASP533			ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533	ASP533
THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718	THR718
LEU721						LEU721						LEU721	
ASN722	ASN722		ASN722		ASN722	ASN722	ASN722				ASN722	ASN722	ASN722
		ARG362		ARG362									
				ARG634									
	ARG488		ARG488		ARG488	ARG488	ARG488		ARG488		ARG488		ARG488
					ILE535		ILE535						
	ILE535		ILE535	ILE535					ILE535		ILE535		
			HIS632					HIS632					ILE535
				GLN633									
				ALA635									
				ALA715									
		GLY717											
								SER722	SER722				
										HIS364	HIS364		
								ALA351					ALA351
ASN352	ASN352		ASN352		ASN352	ASN352	ASN352	ASN352	ASN352	ASN352	ASN352		ASN352
	GLU356	GLU356	GLU356		GLU356	GLU356	GLU356	GLU356		GLU356	GLU356	GLU356	GLU356
			THR426		TYR426								TYR426
													ILE427
PTR723	PTR723		PTR723		PTR723	PTR723	PTR723	PTR723	PTR723		PTR723	PTR723	PTR723
	LYS354										ILE355		
							LYS354		LYS354		LYS354		
			GLY533										
			TYR416										
		LYS374				LYS374		LYS374		LYS374			
		GLY363		GLY363									
				TYR268									
				GLY365									

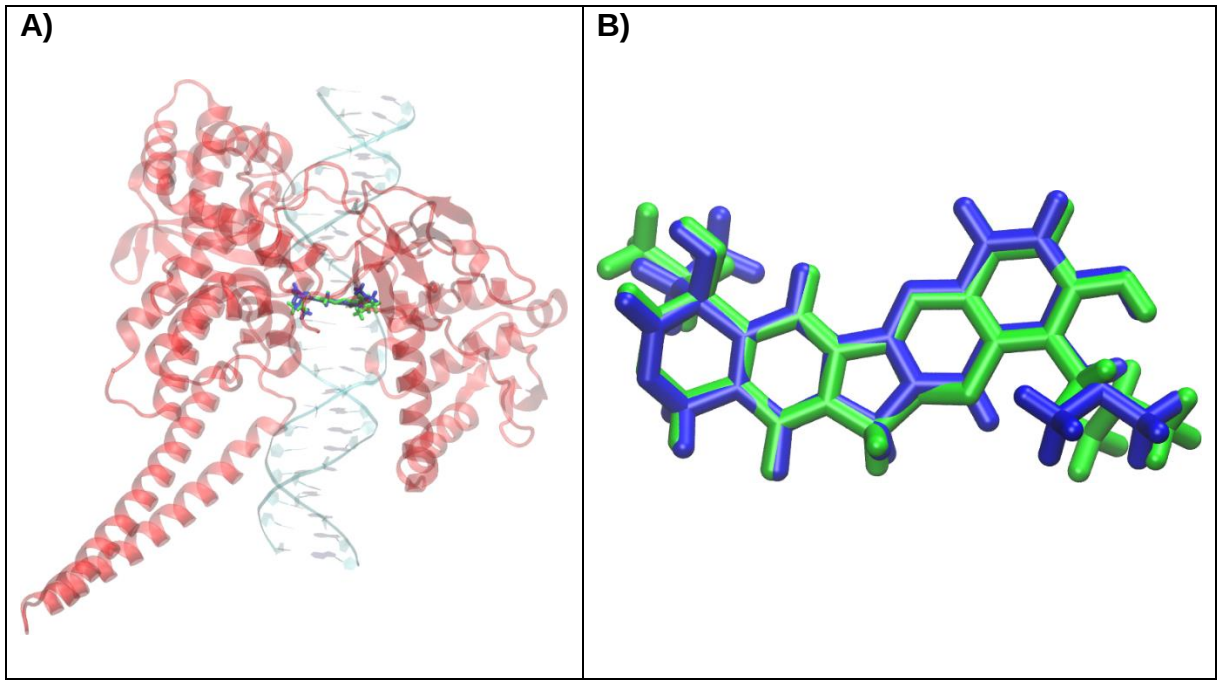


Figure **S1.** Comparison of between the crystal pose (blue) and the docked pose (green) of topotecan. The RMSD of heavy atoms is 1.0 Å.

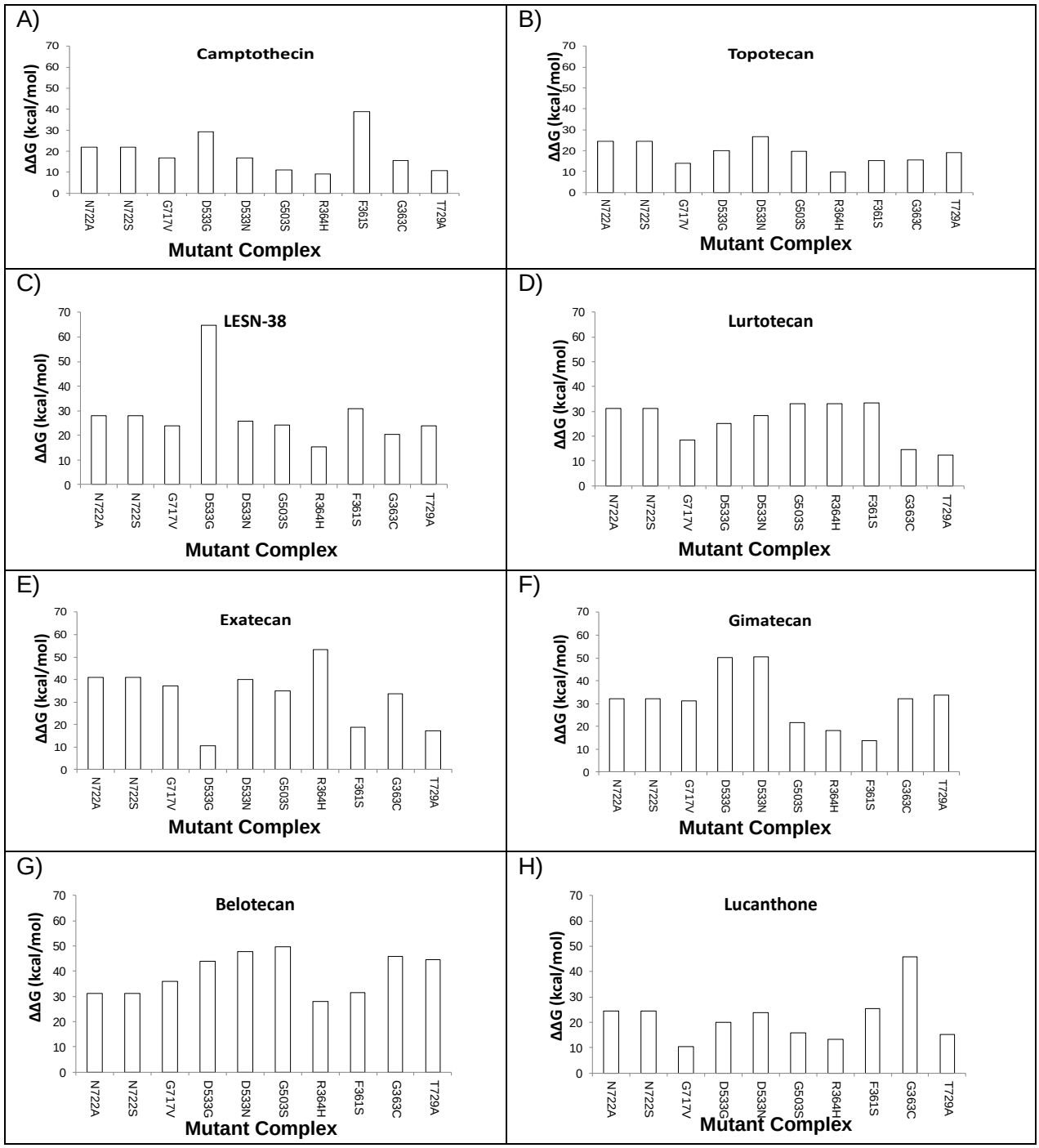


Figure **S2.** Change in binding energy (ΔΔG) of Top I inhibitors to mutants

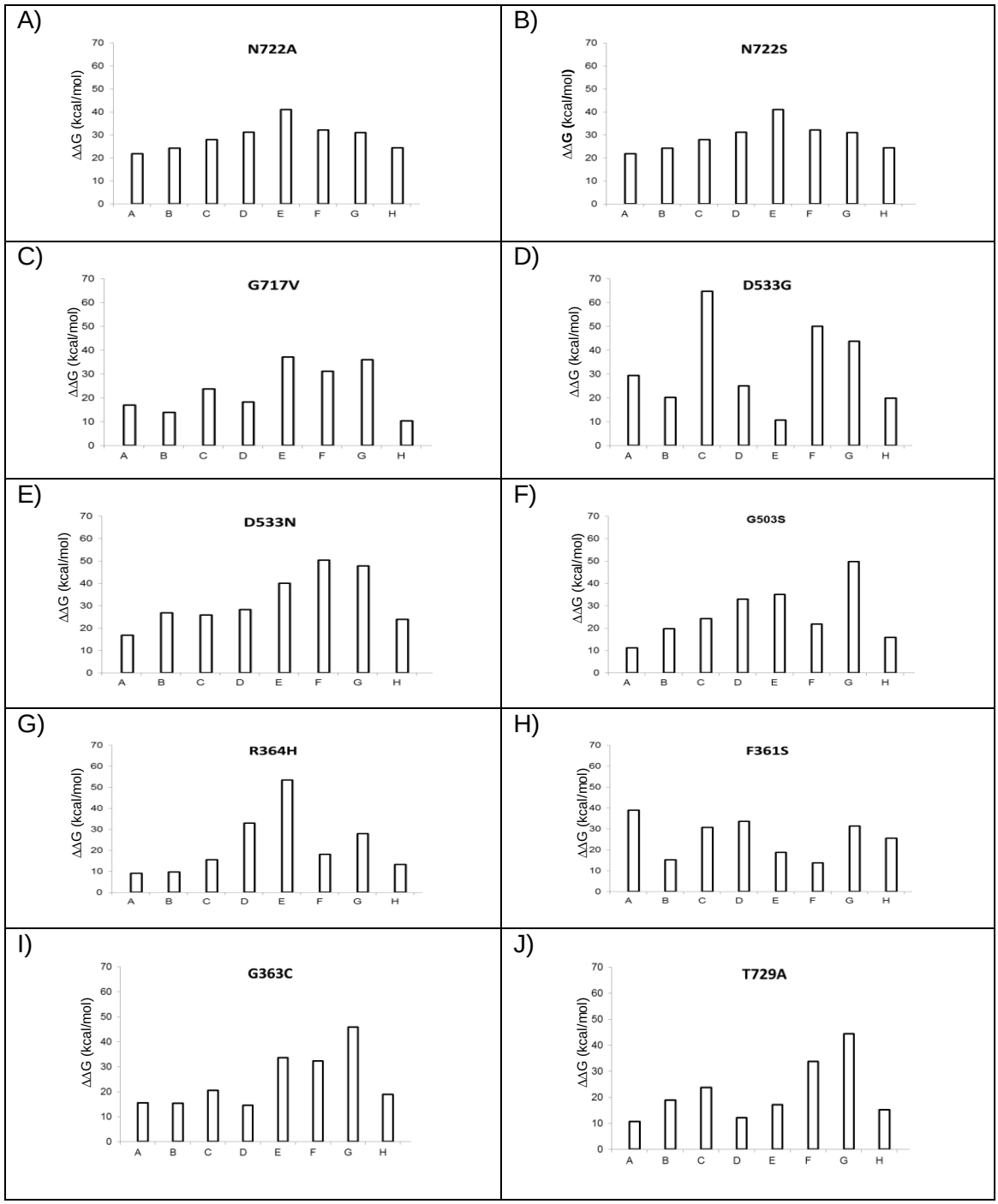
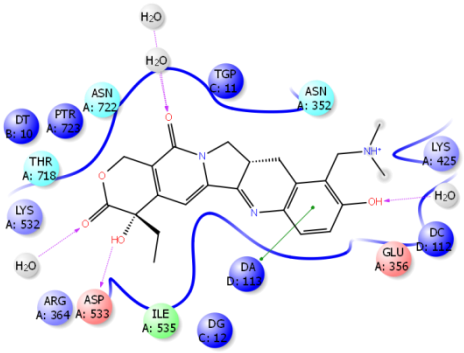
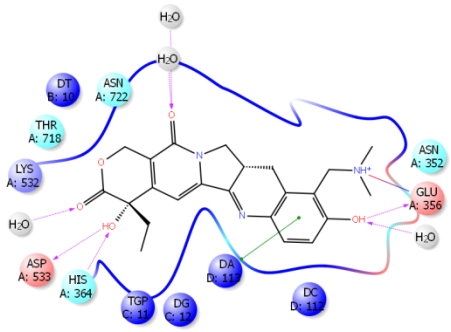
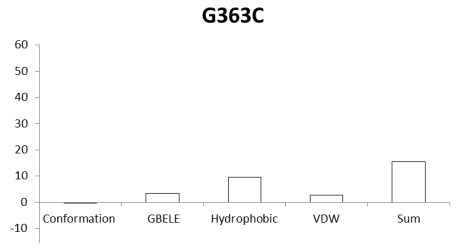
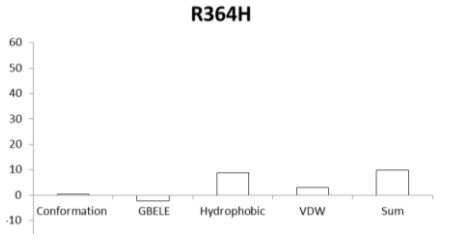
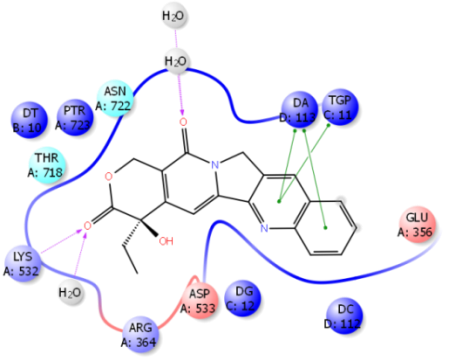
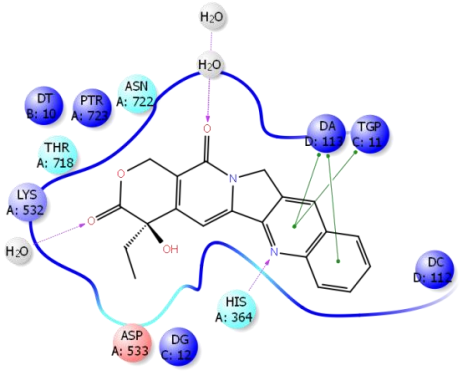
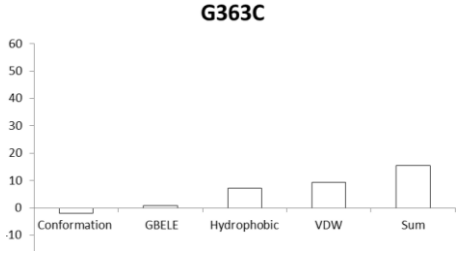
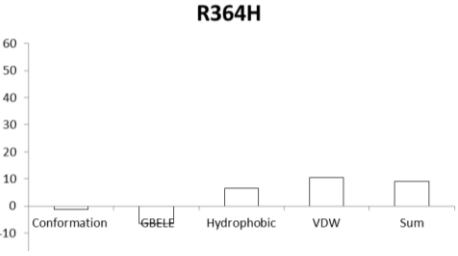
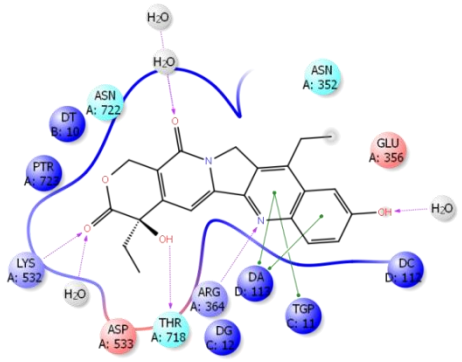
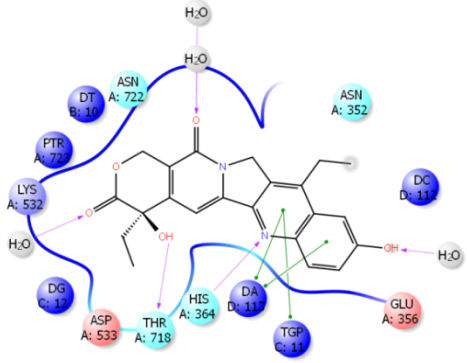
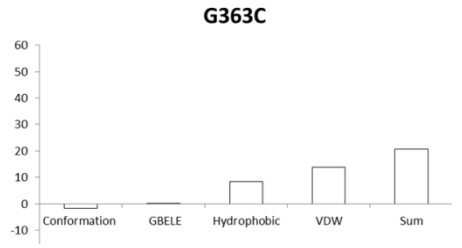
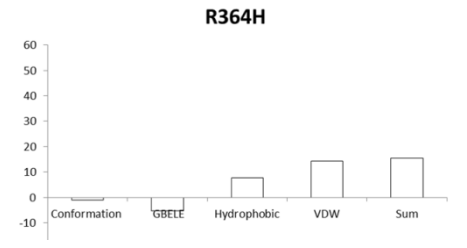
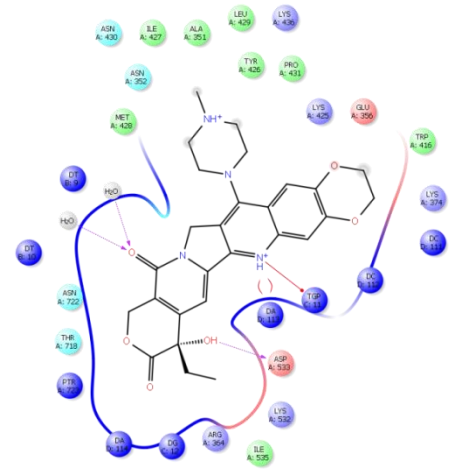
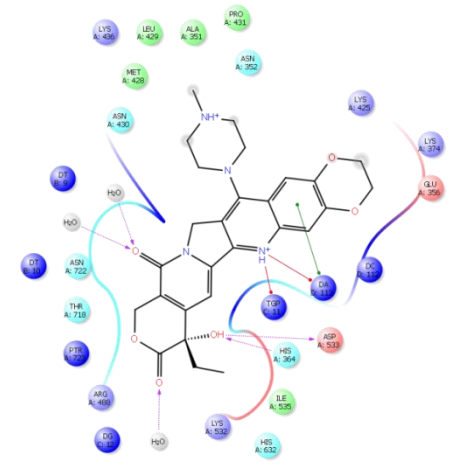
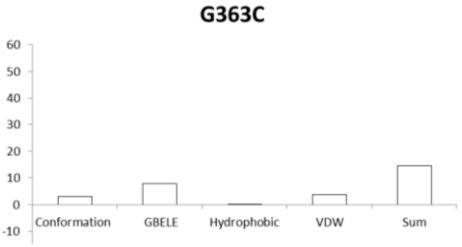
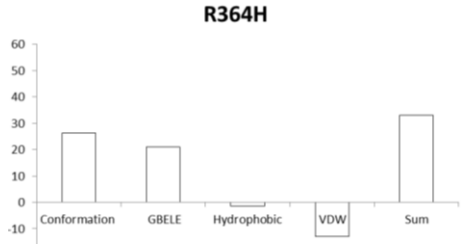
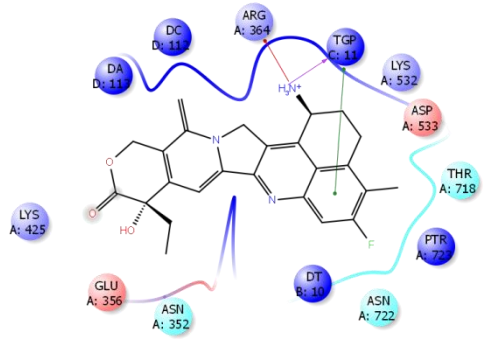
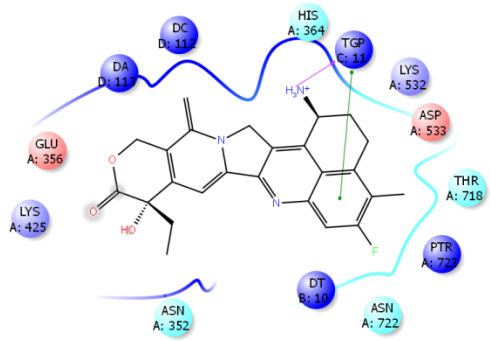
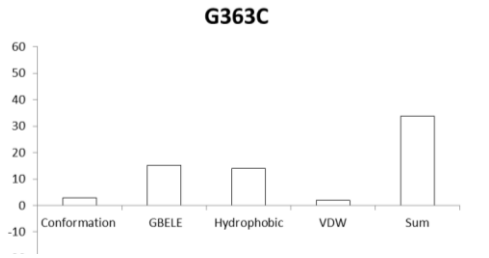
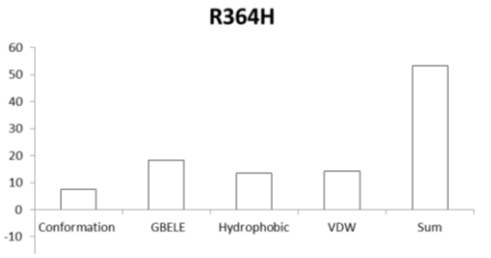
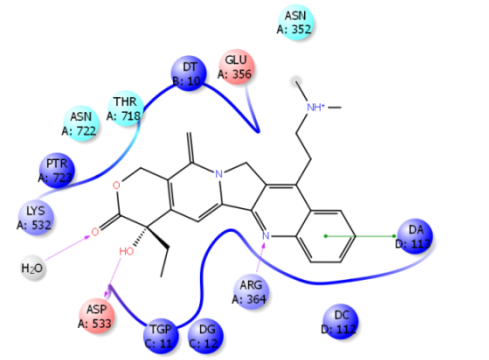
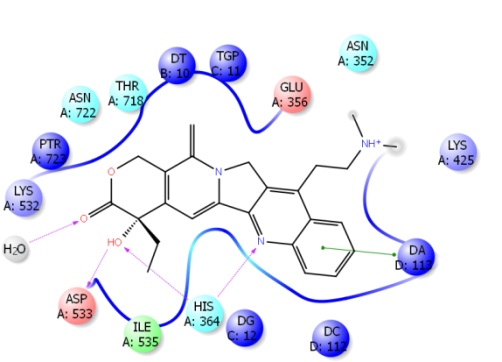
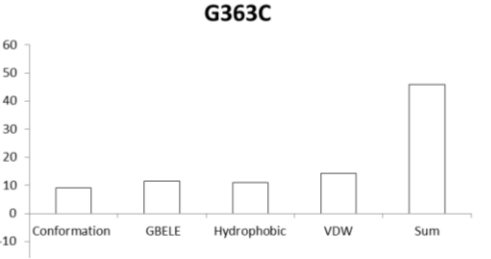
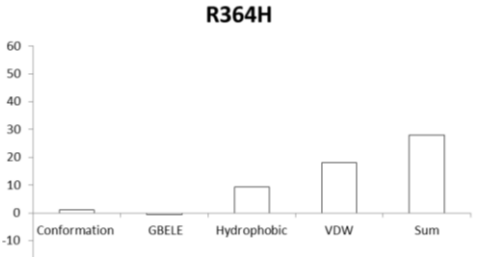


Figure **S3.** Change in binding energy (ΔΔG) of Top I inhibitors A-H bound to mutated complexes**. A)** Camptothecin **B)** Topotecan **C)** SN-38 **D)** Lurtotecan **E)** Exatecan **F)** Gimatecan **G)** Belotecan **H**) Lucanthone

	G363C	R364H
Topotecan		
ΔΔG Decomposition		
Camptothecin		
ΔΔG Decomposition		

SN38		
ΔΔG Decomposition		
Lurtotecan		
ΔΔG Decomposition		

Exatecan		
ΔΔG Decomposition		
Belotecan		
ΔΔG Decomposition		

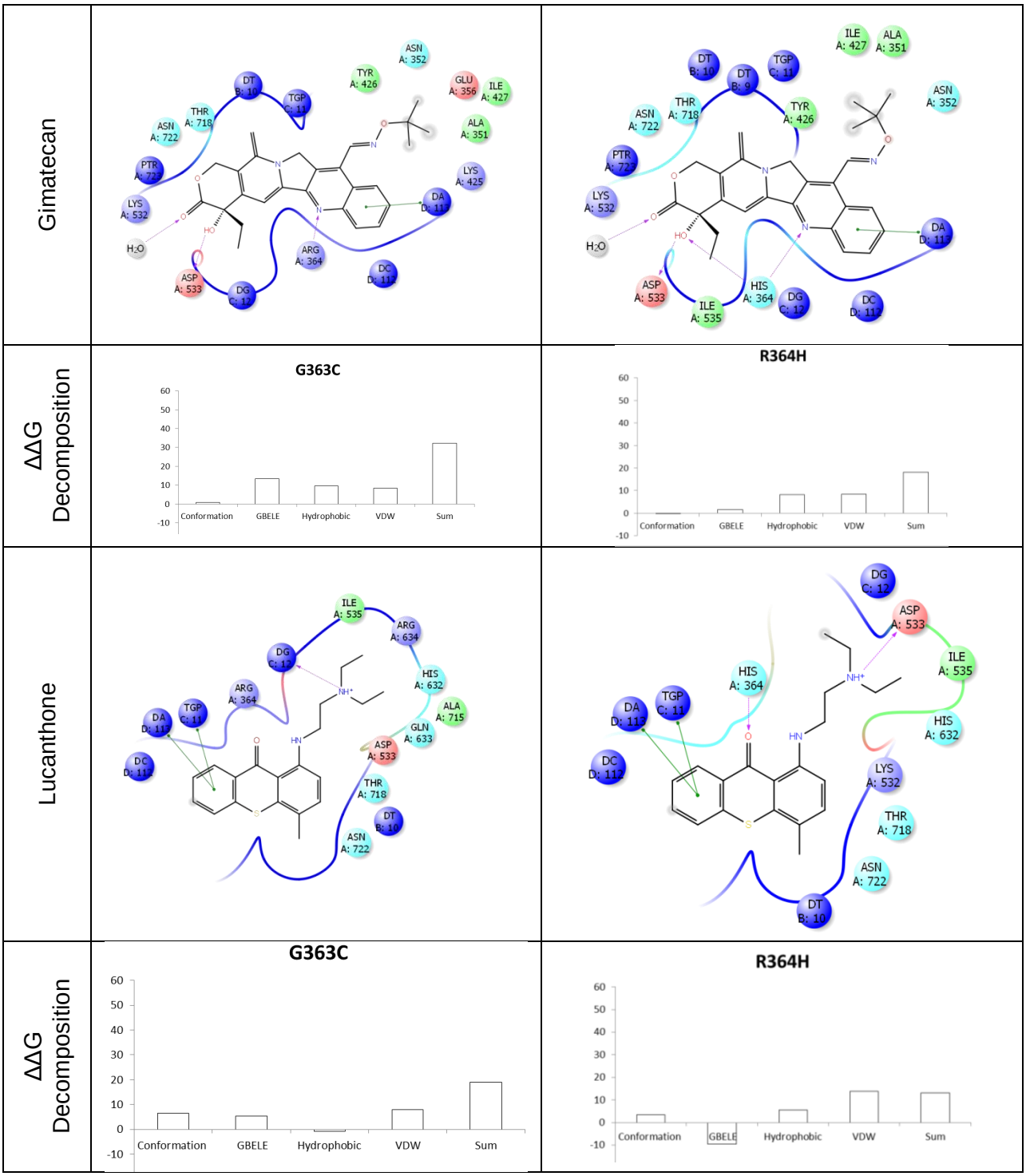


Figure **S4**. Comparison of mutated complexes G363C and R364H

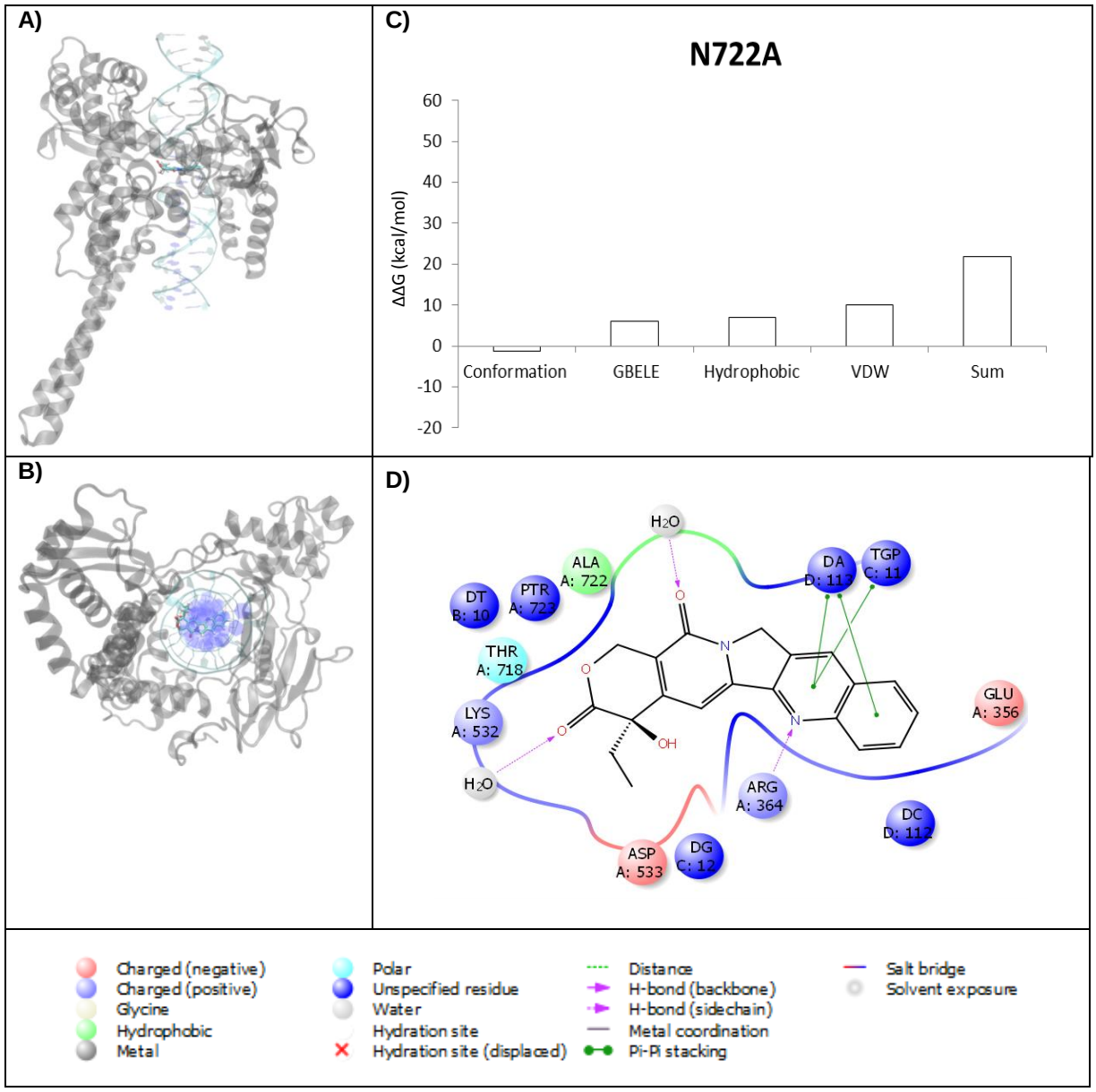


Figure **S5.** Camptothecin Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

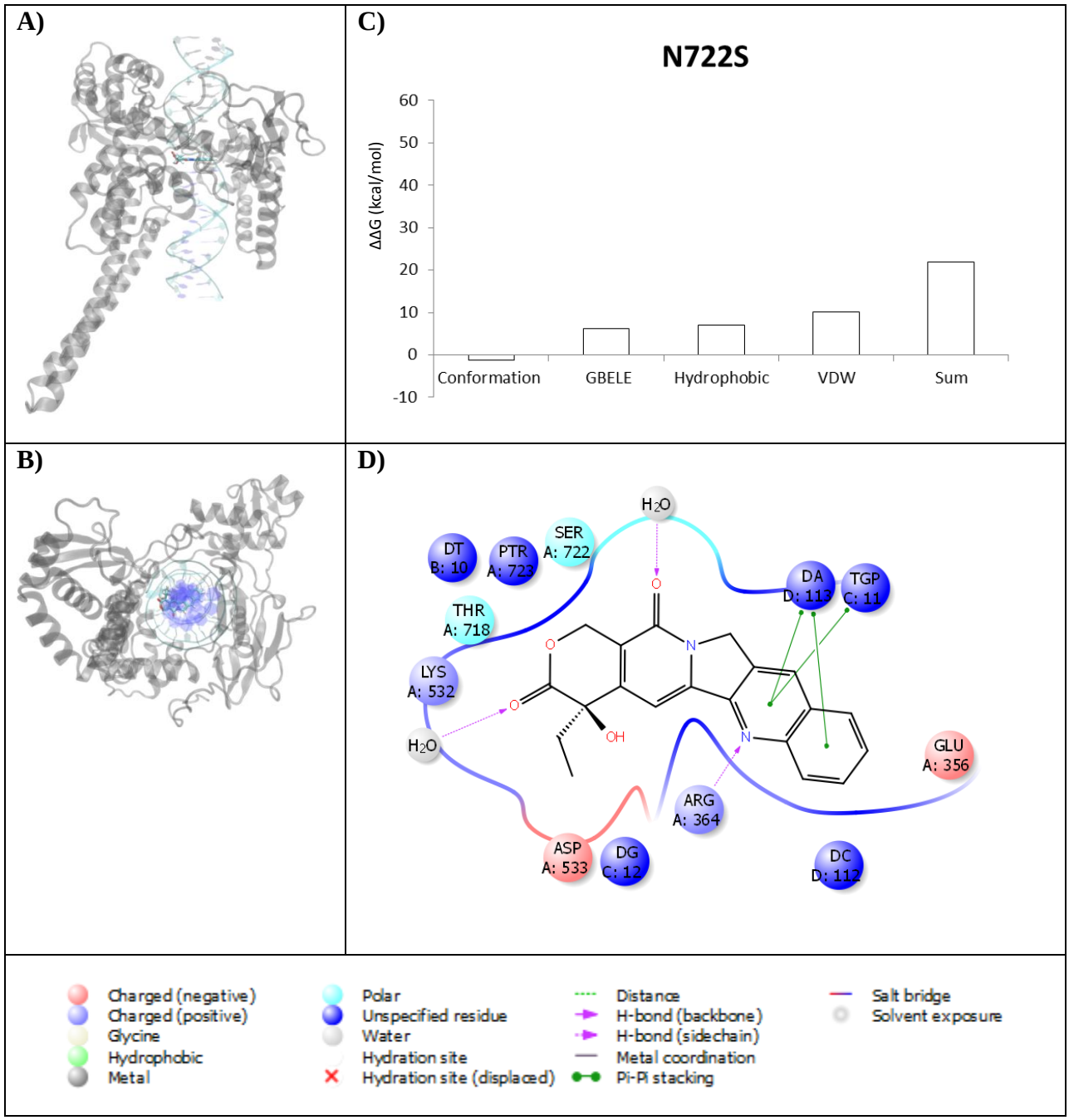


Figure **S6.** Camptothecin Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

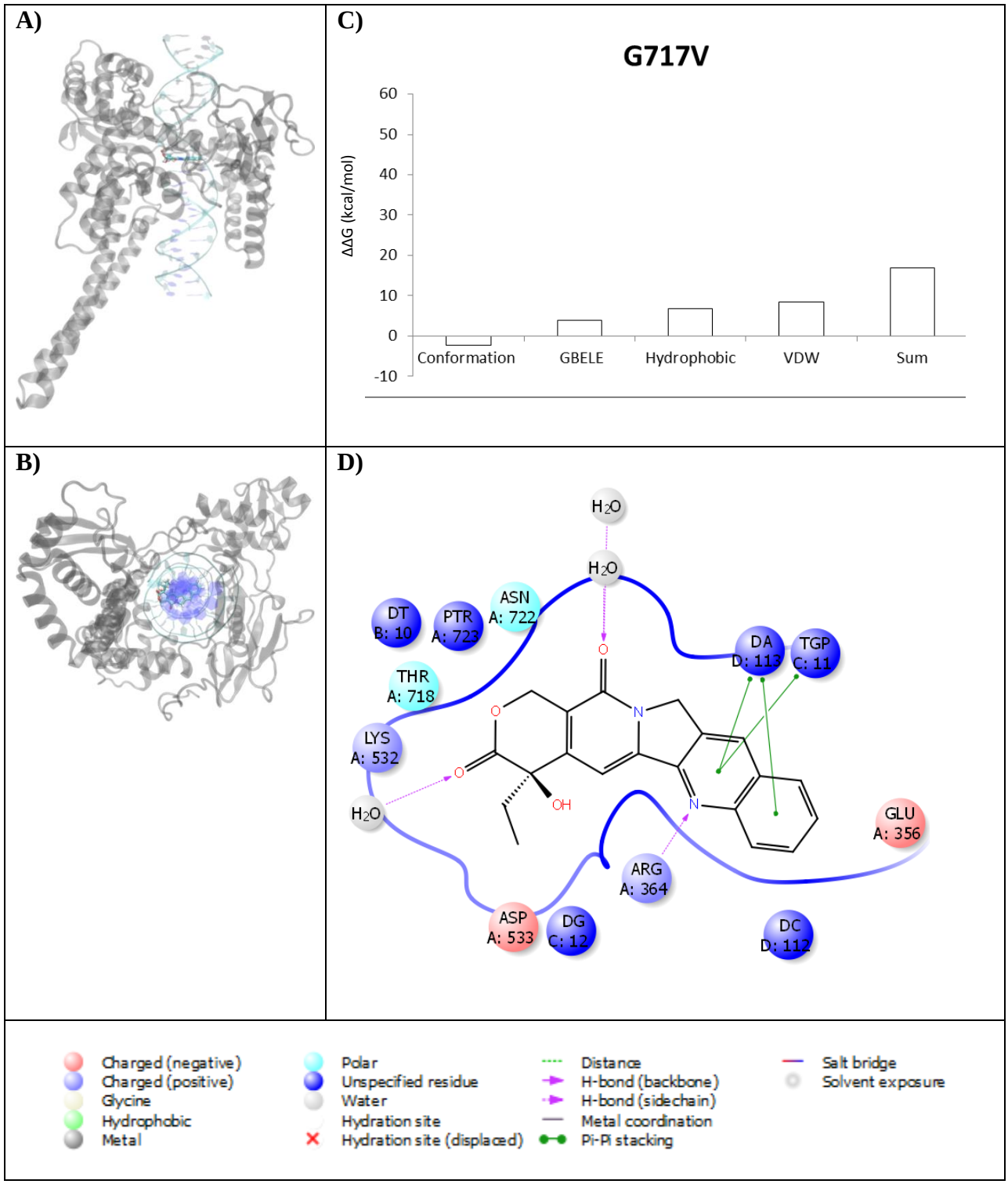


Figure **S7.** Camptothecin Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

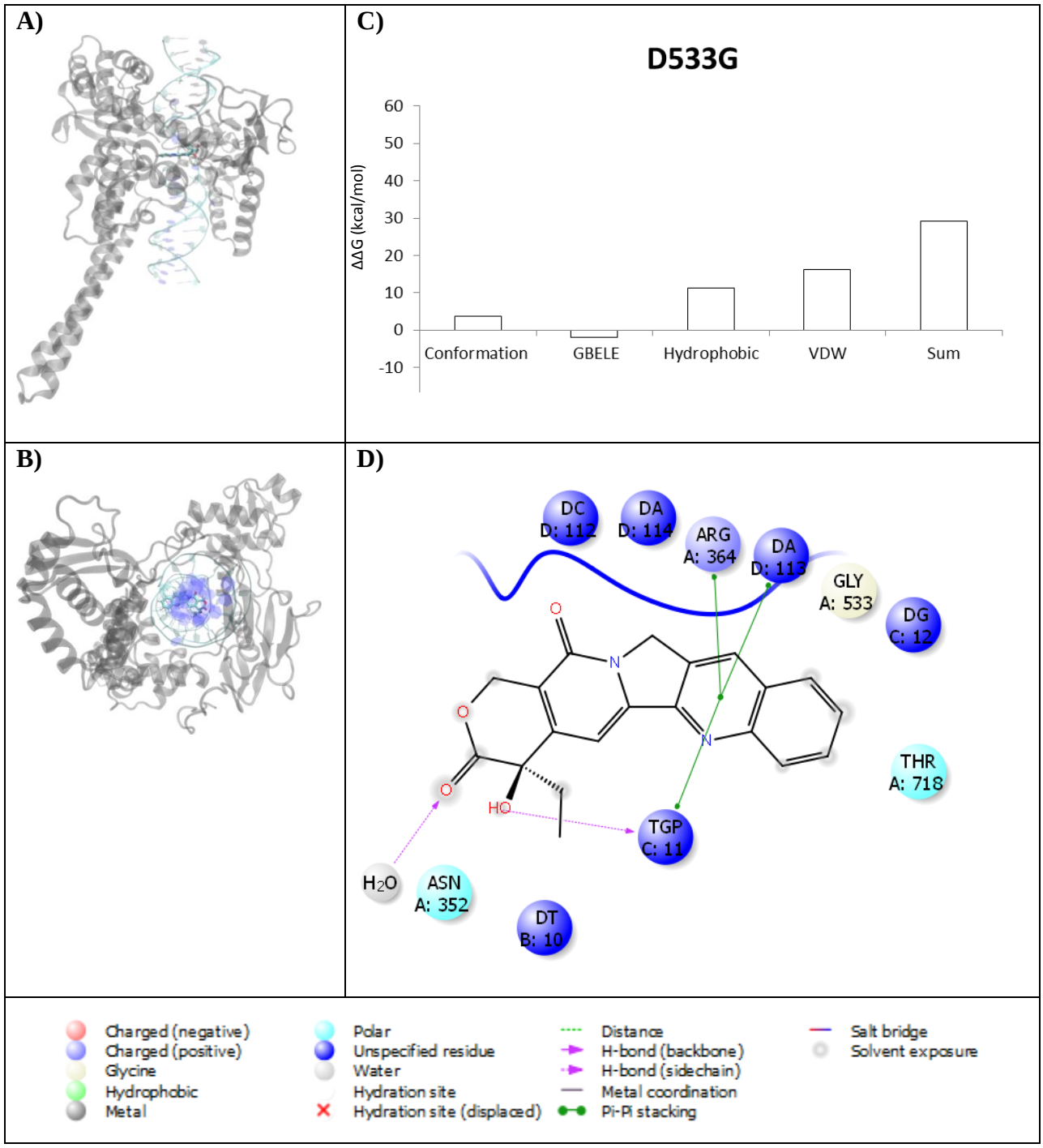


Figure **S8.** Camptothecin Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

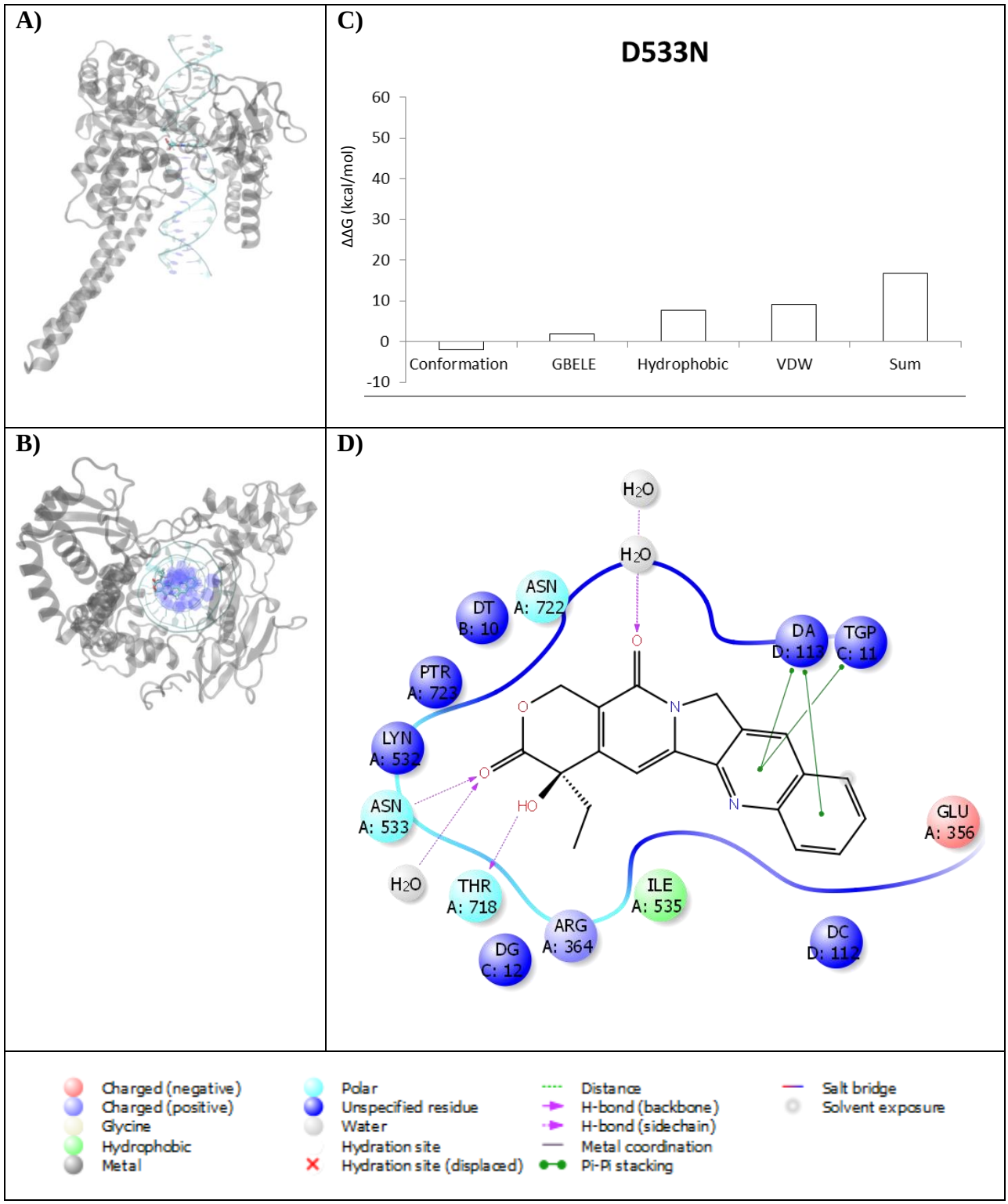


Figure **S9.** Camptothecin Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

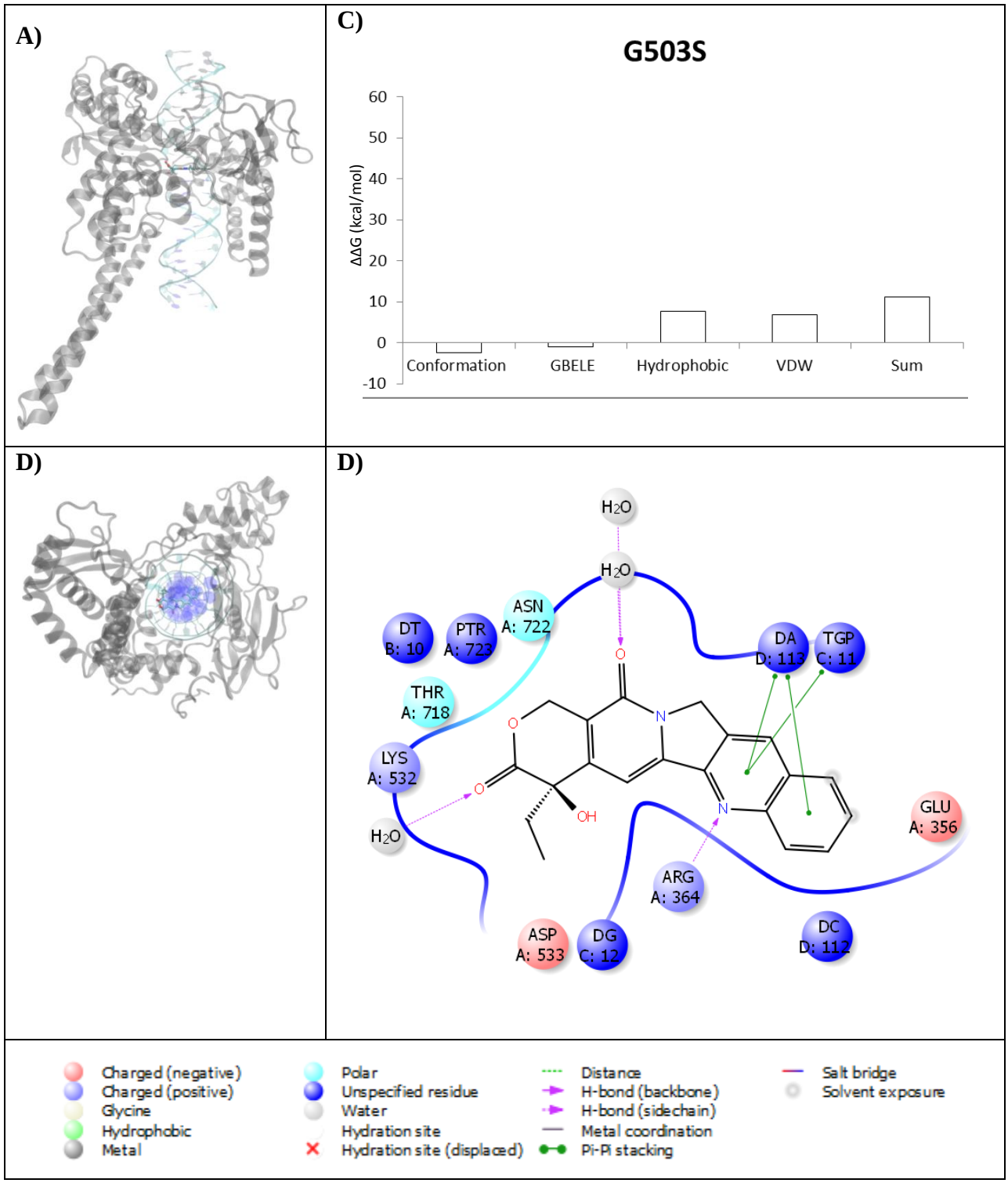


Figure **S10.** Camptothecin Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

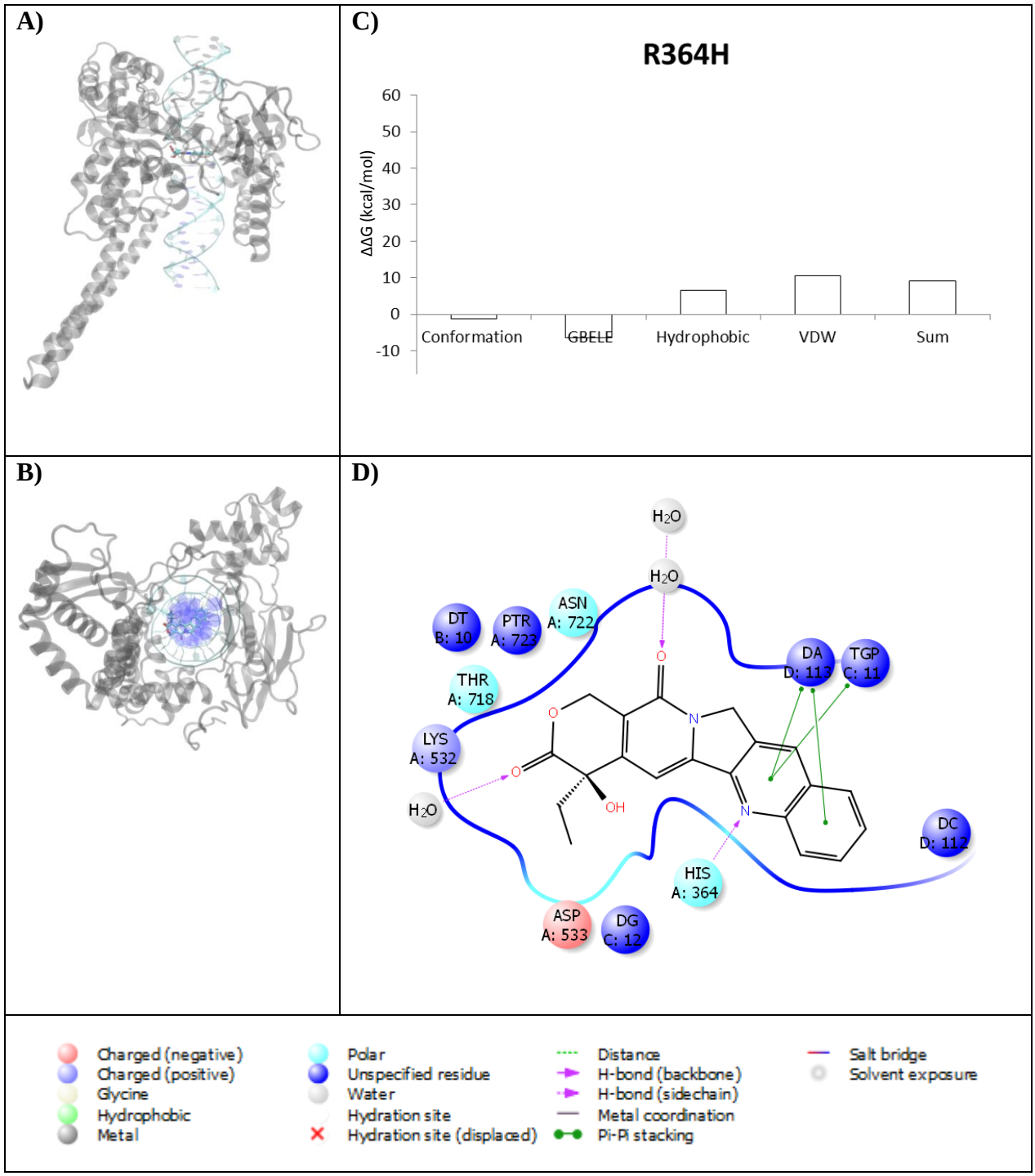


Figure **S11**. Camptothecin Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

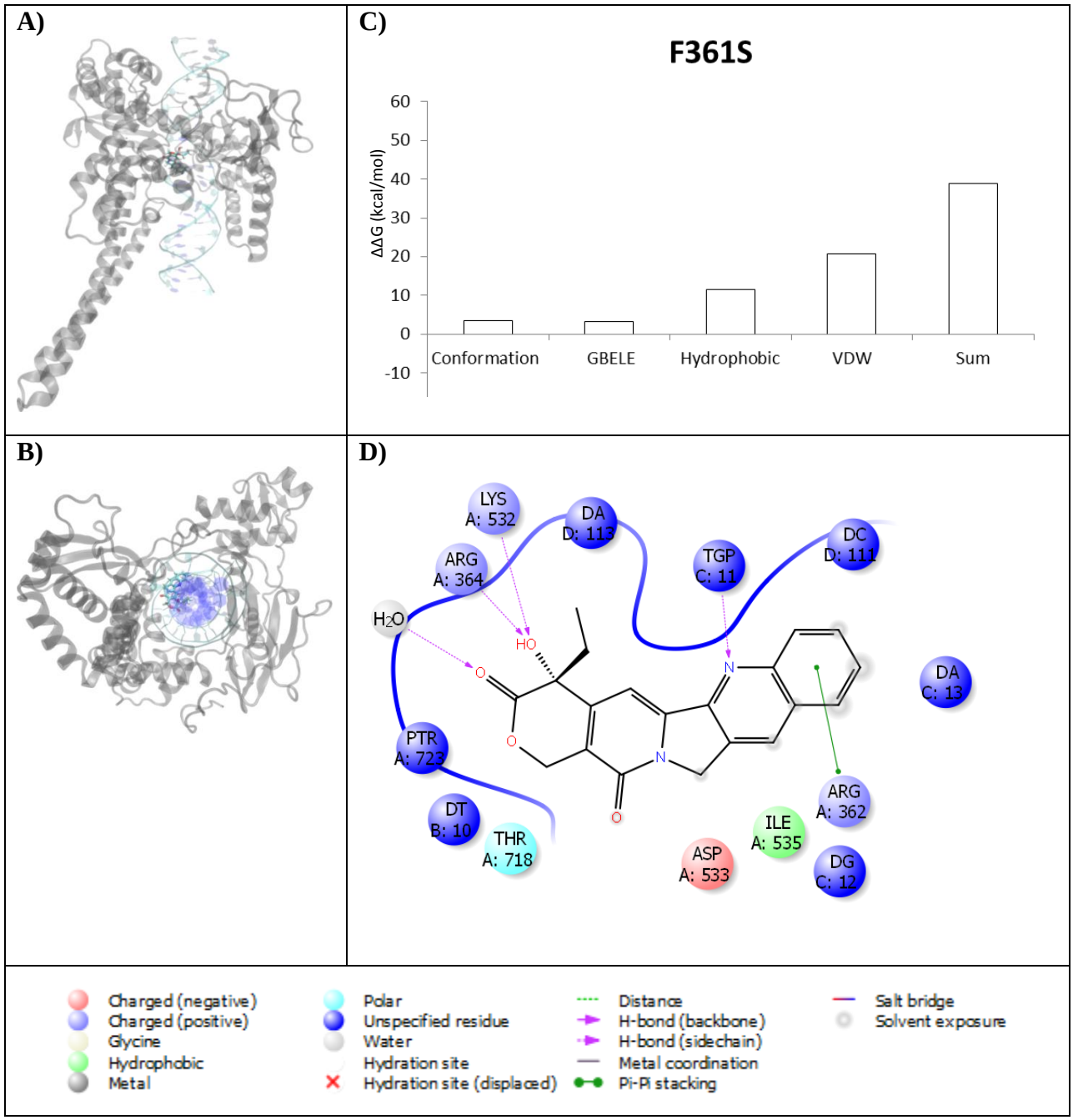


Figure **S12.** Camptothecin Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

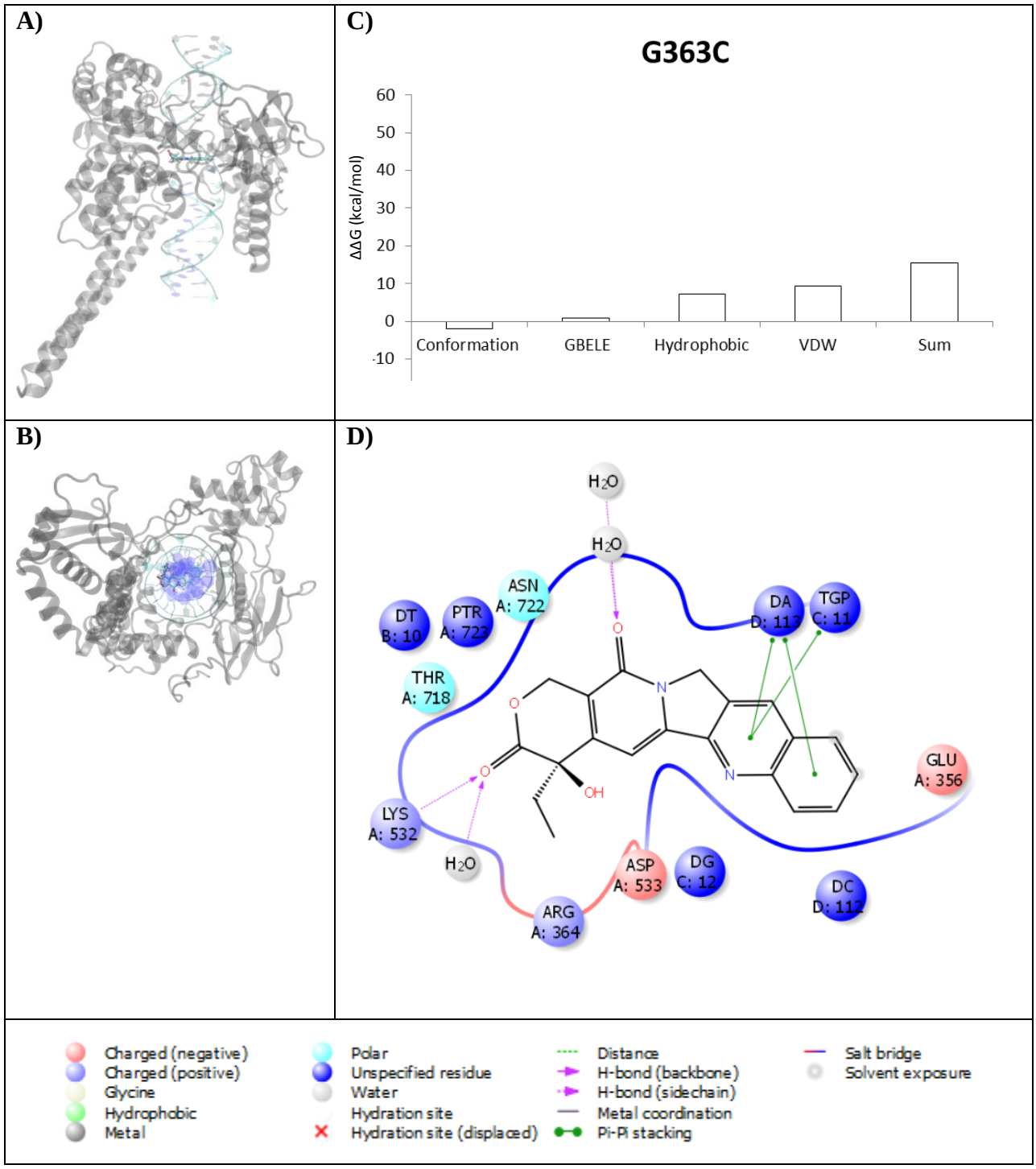


Figure **S13.** Camptothecin Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

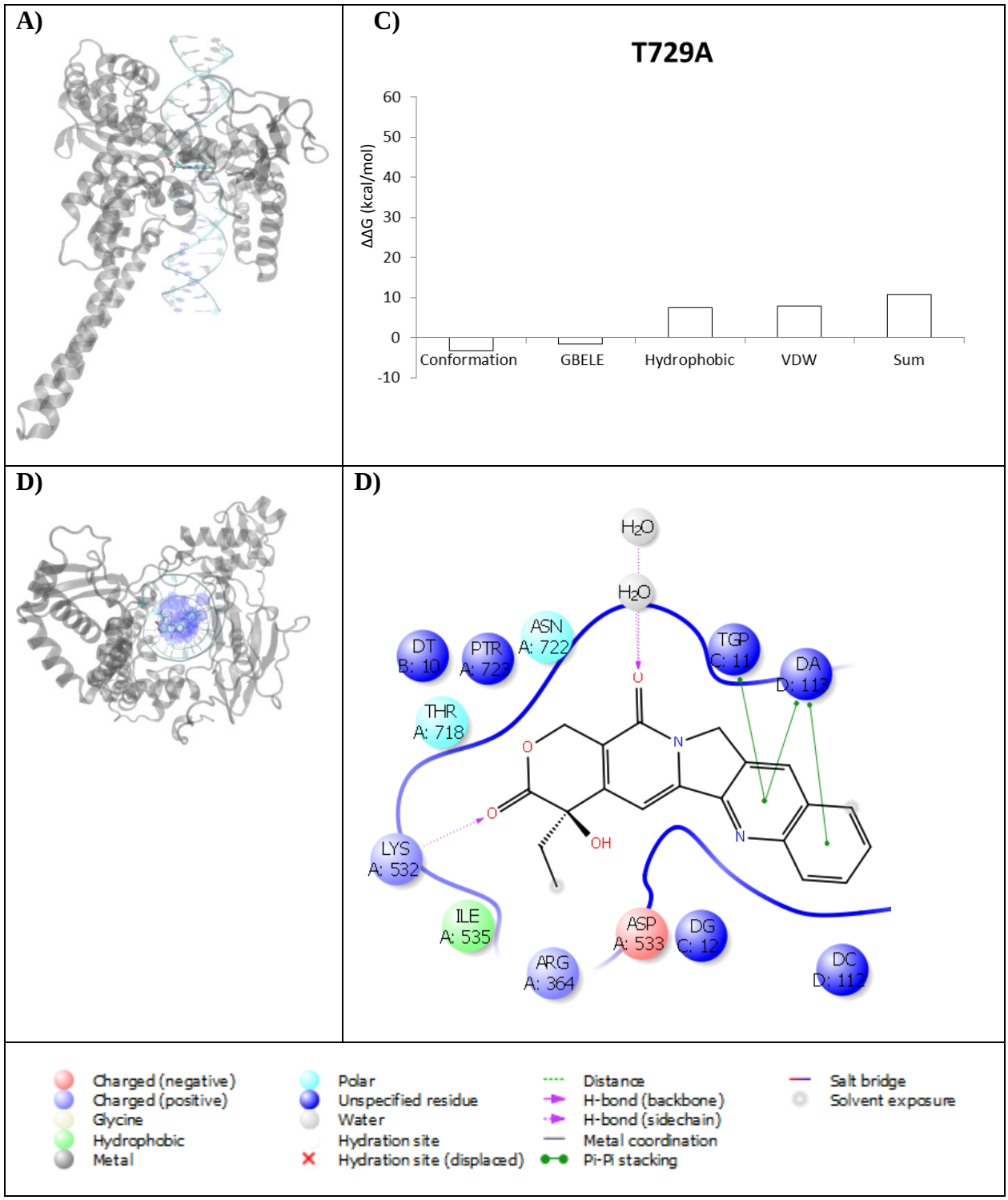


Figure **S14**. Camptothecin Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

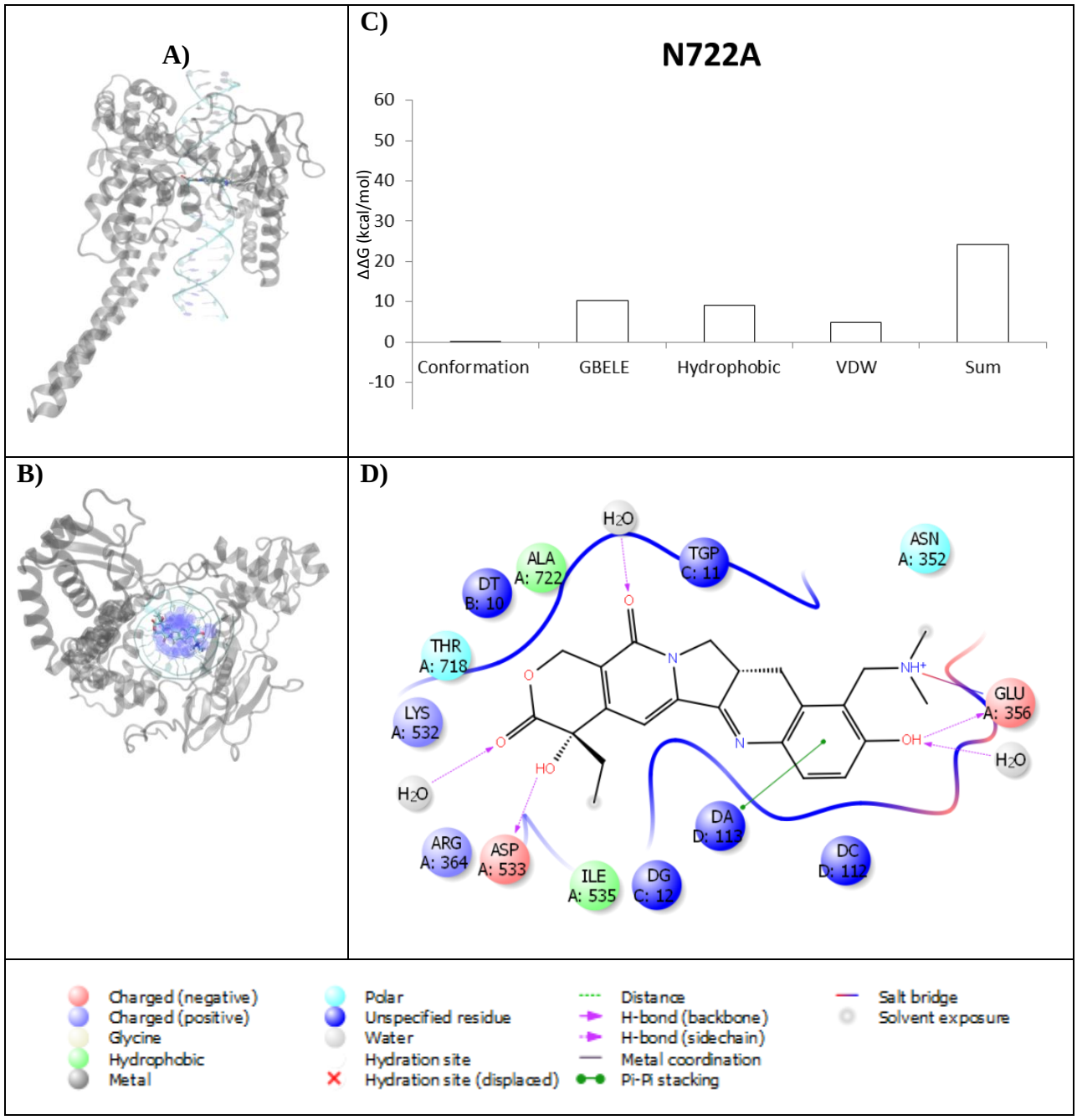


Figure **S15**. Topotecan Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

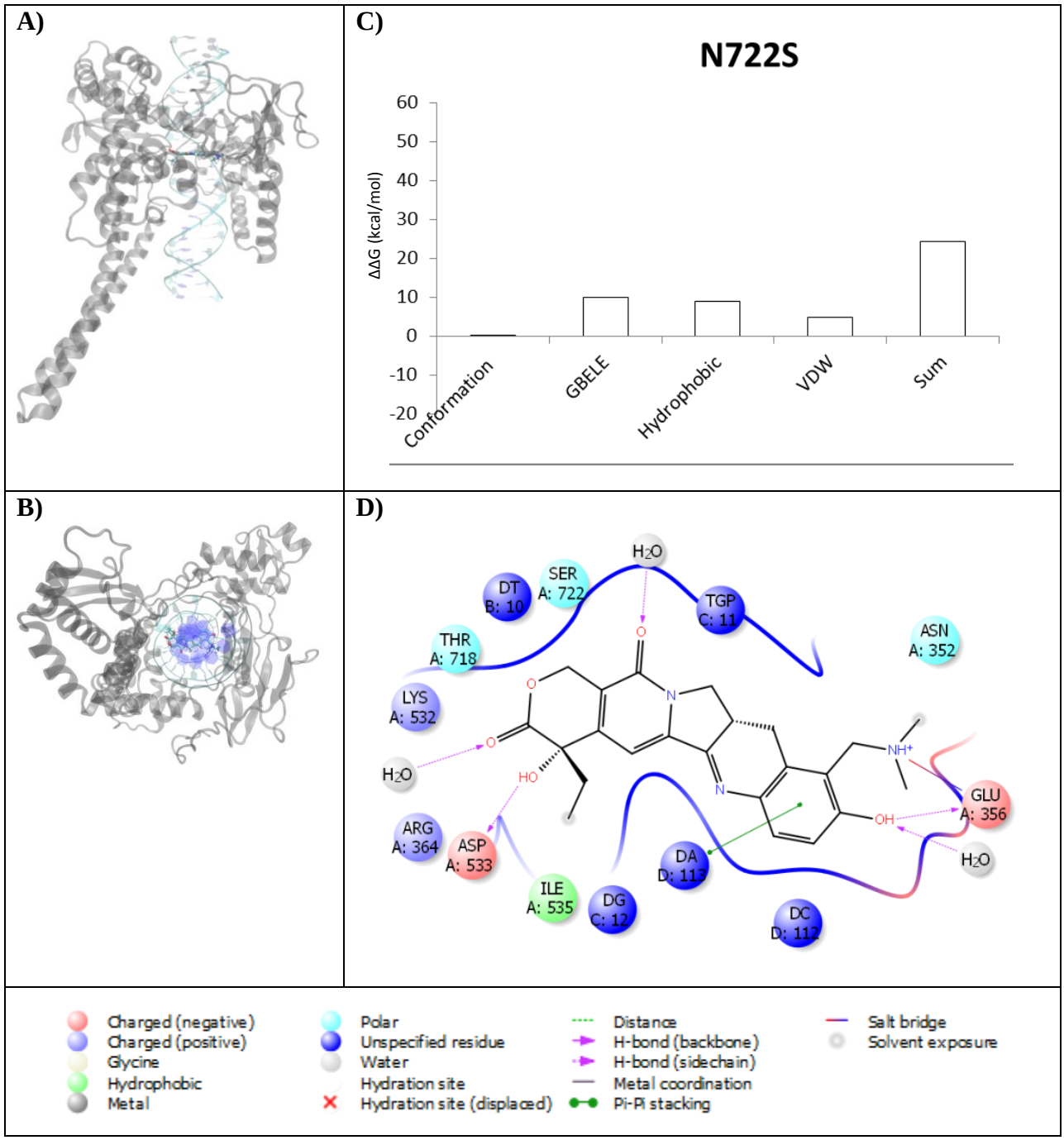


Figure **S16**. Topotecan Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

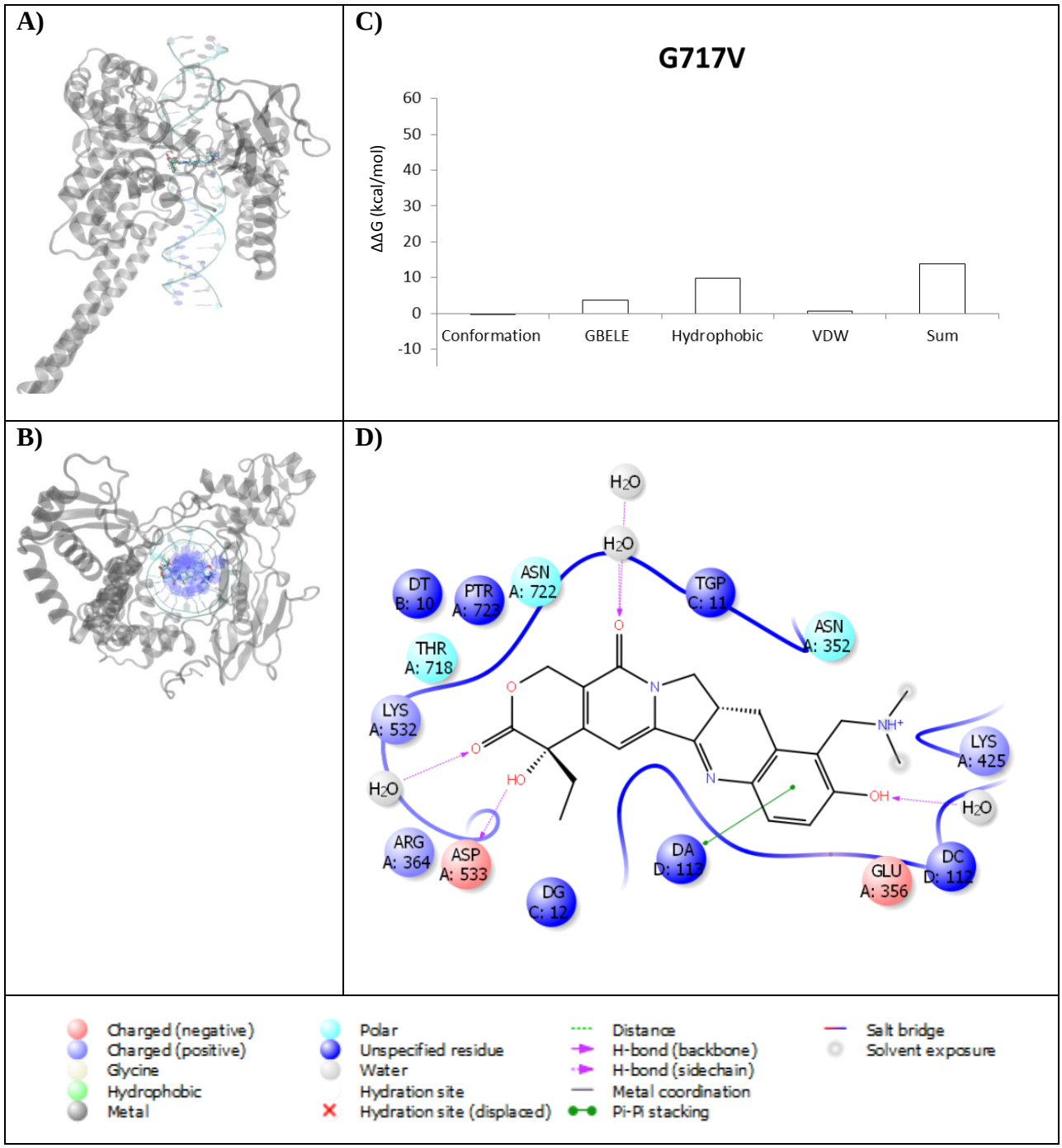


Figure **S17**. Topotecan Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

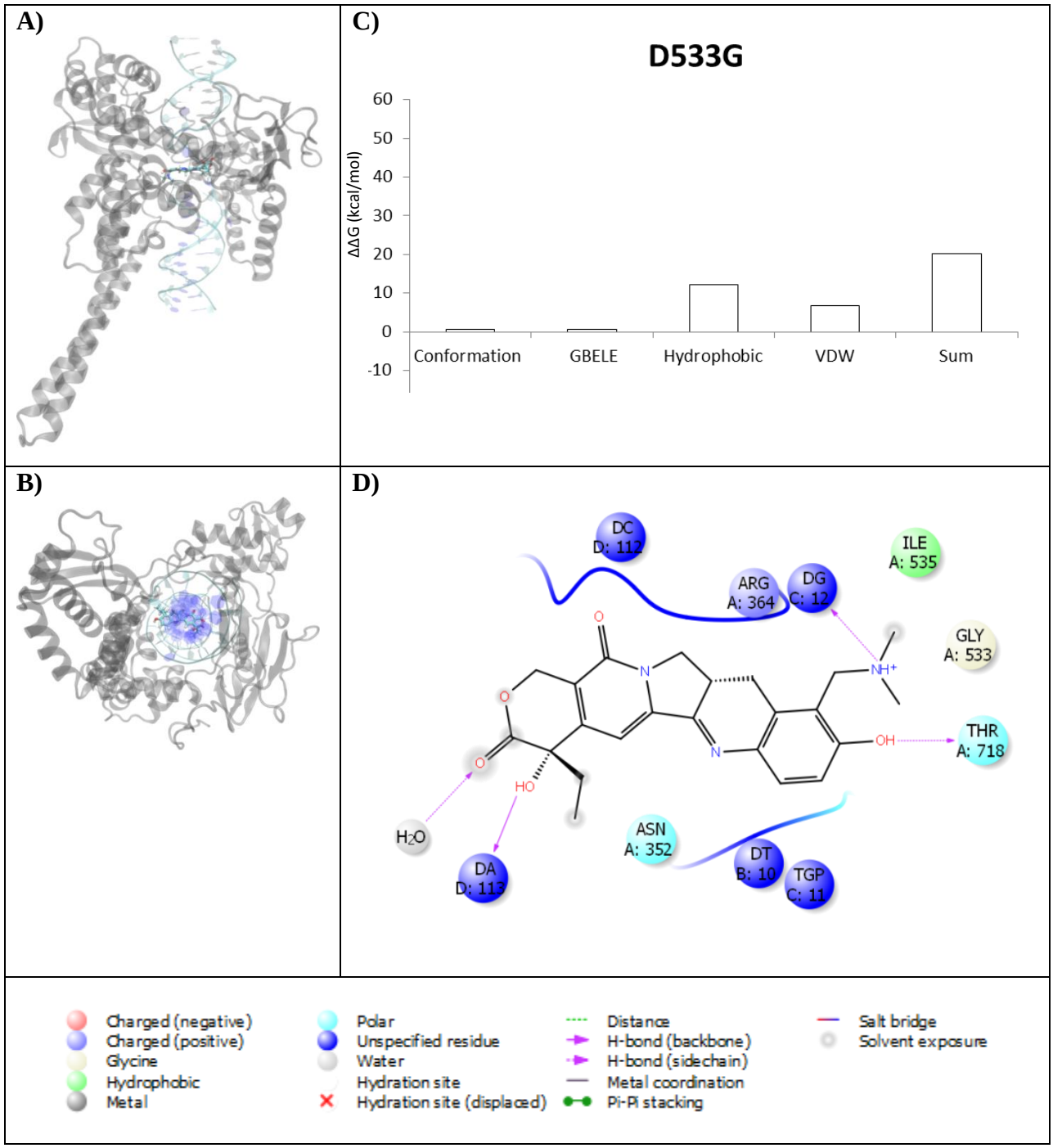


Figure **S18**. Topotecan Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

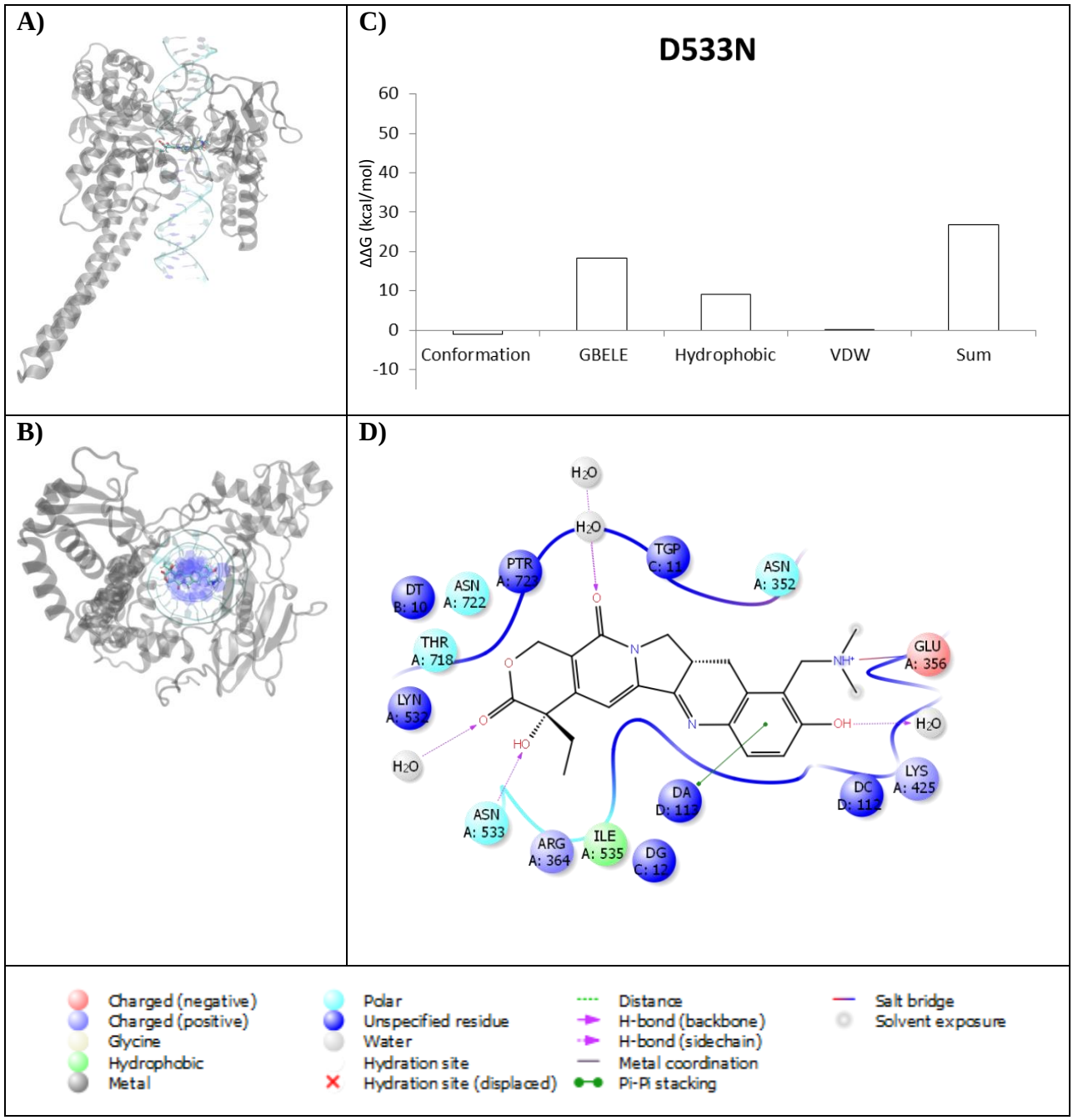


Figure **S19**. Topotecan Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

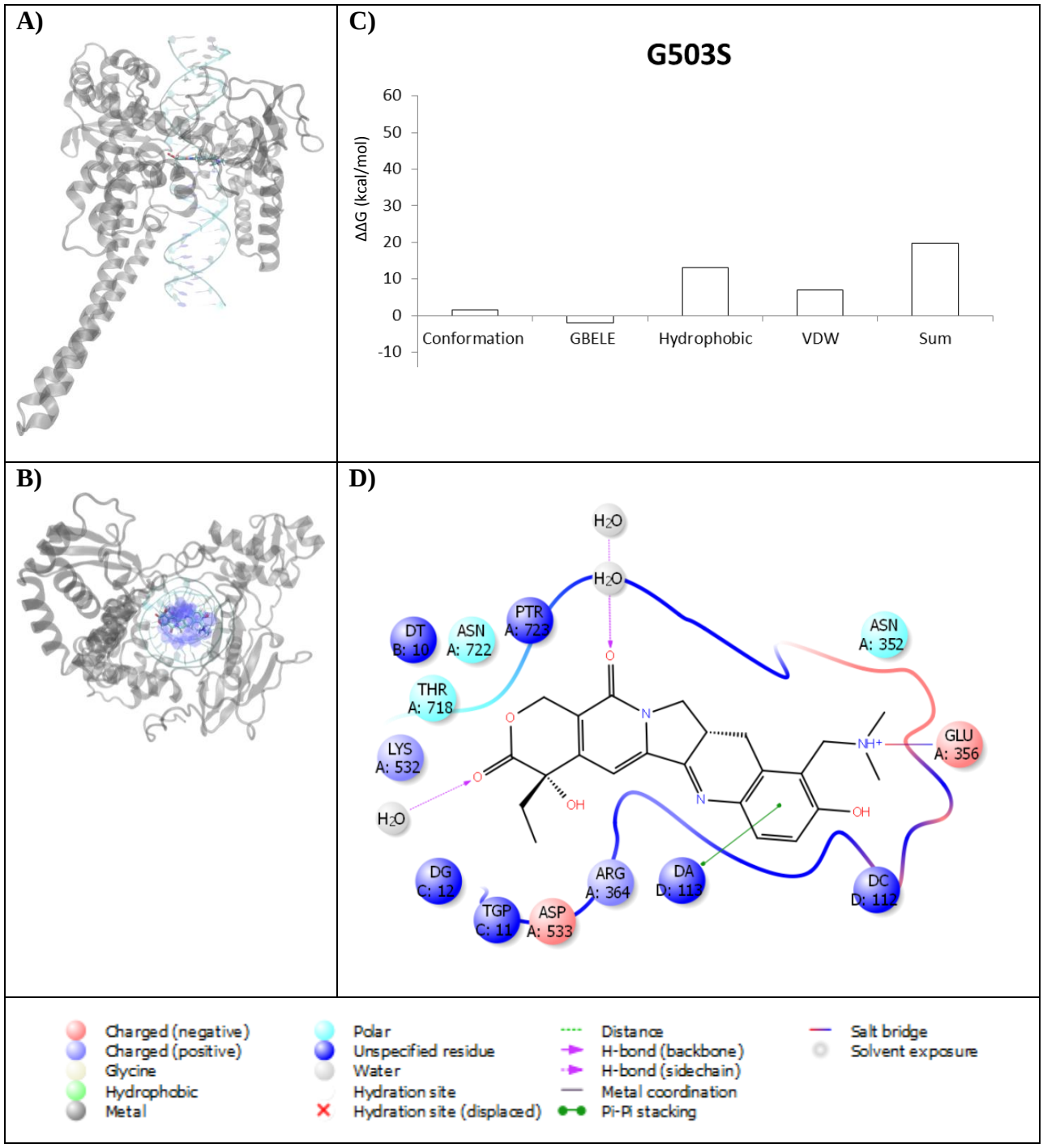


Figure **S20**. Topotecan Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

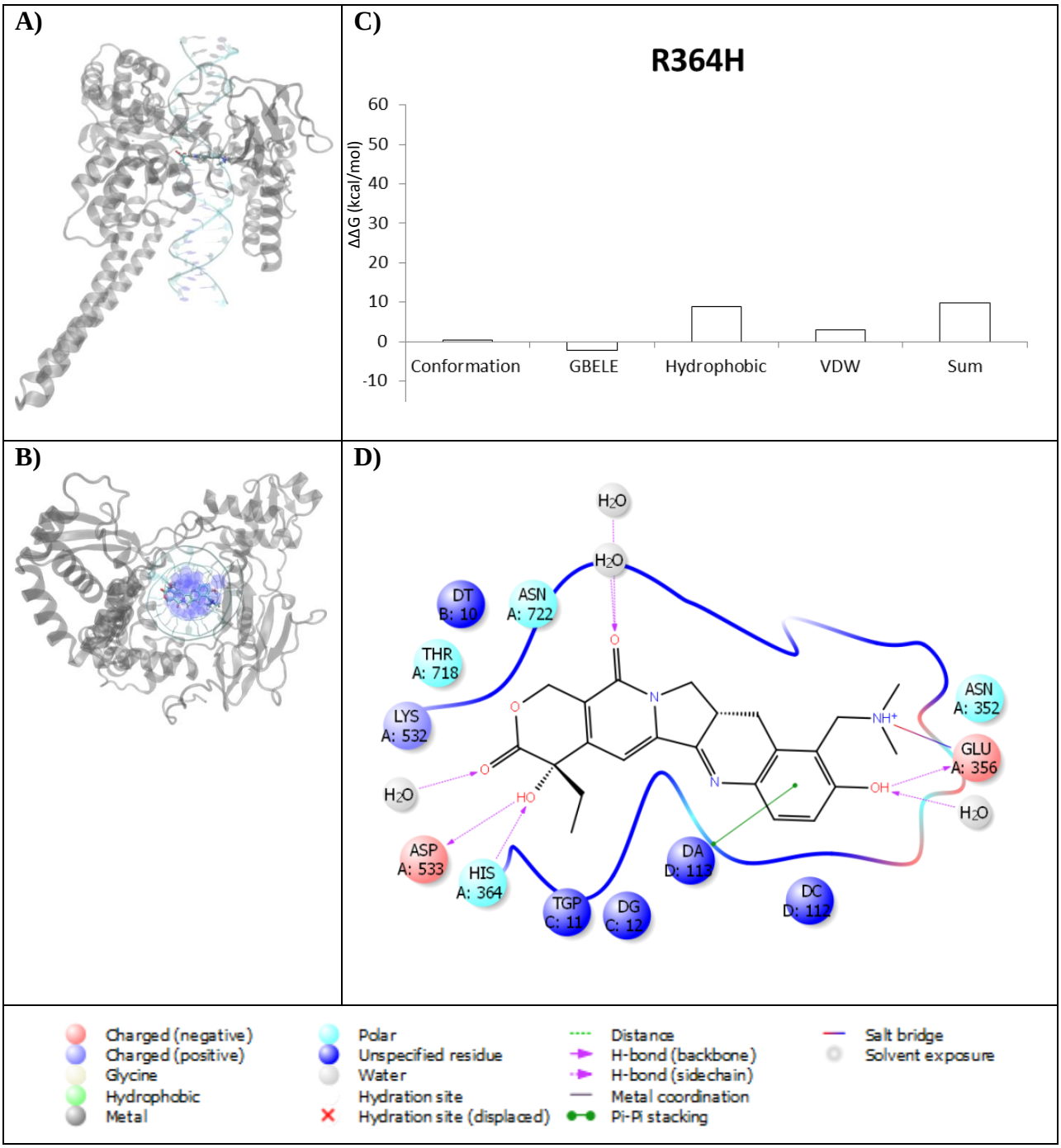


Figure **S21**. Topotecan Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

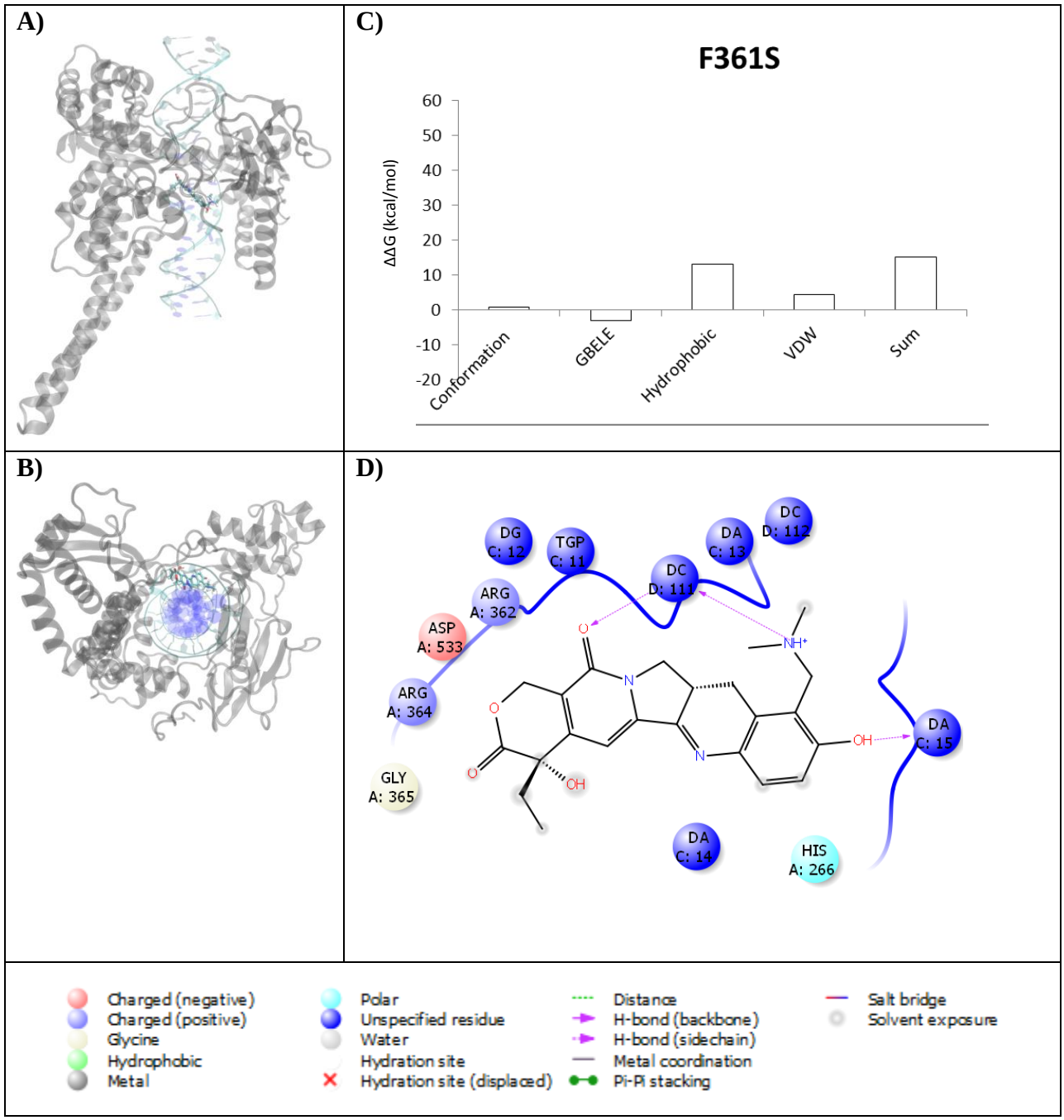


Figure **S22**. Topotecan Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

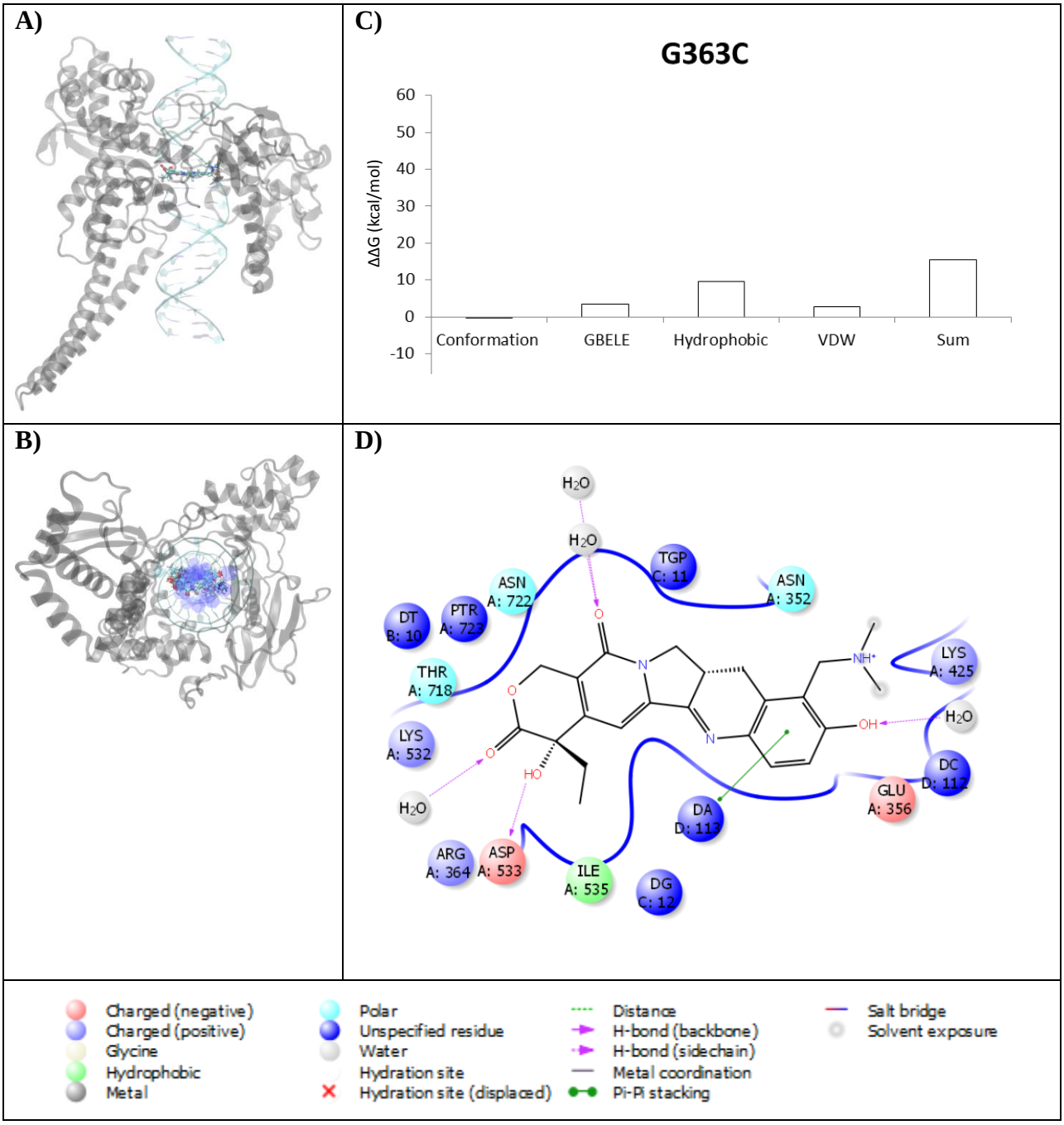


Figure **S23**. Topotecan Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

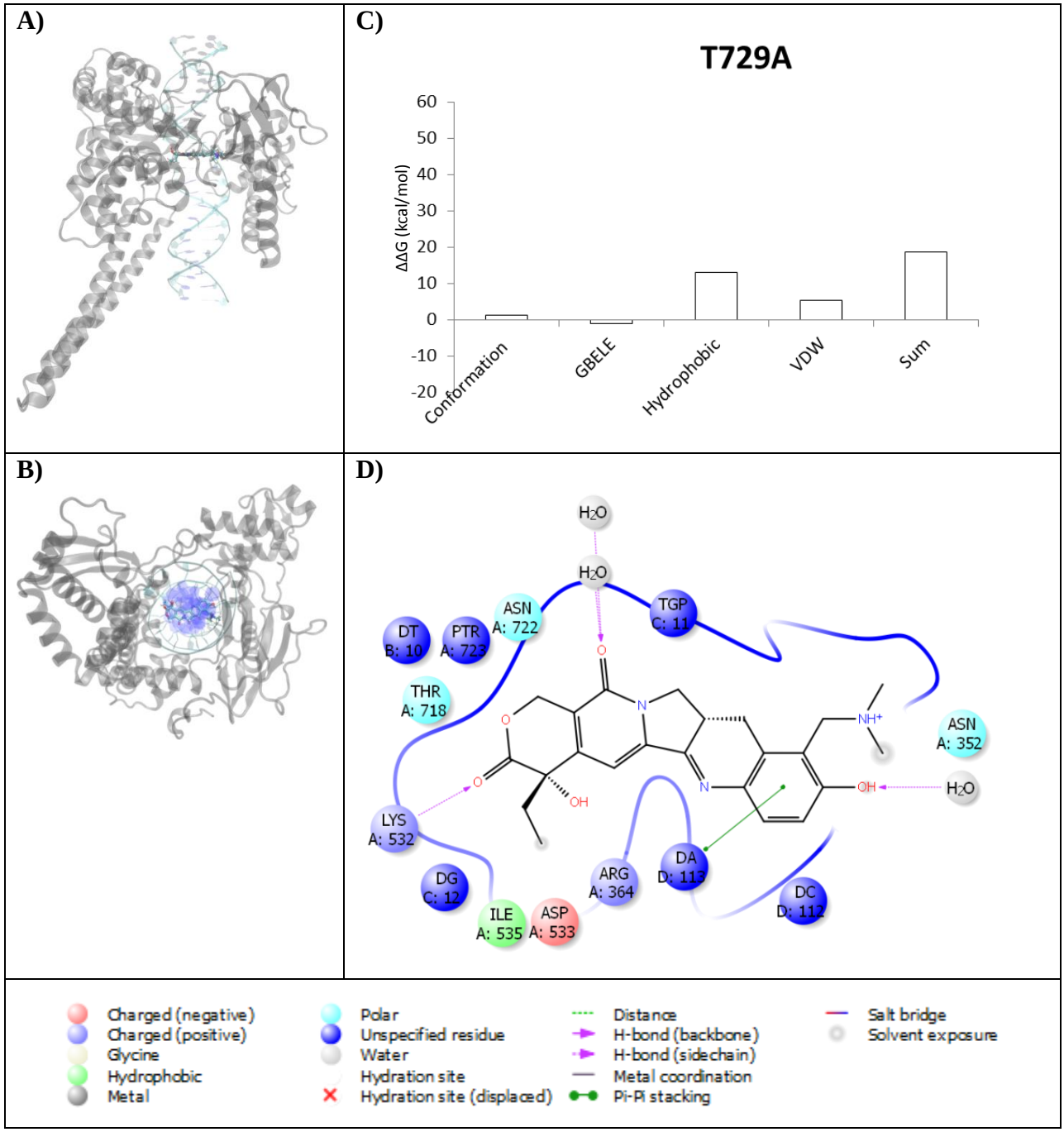


Figure **S24**. Topotecan Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

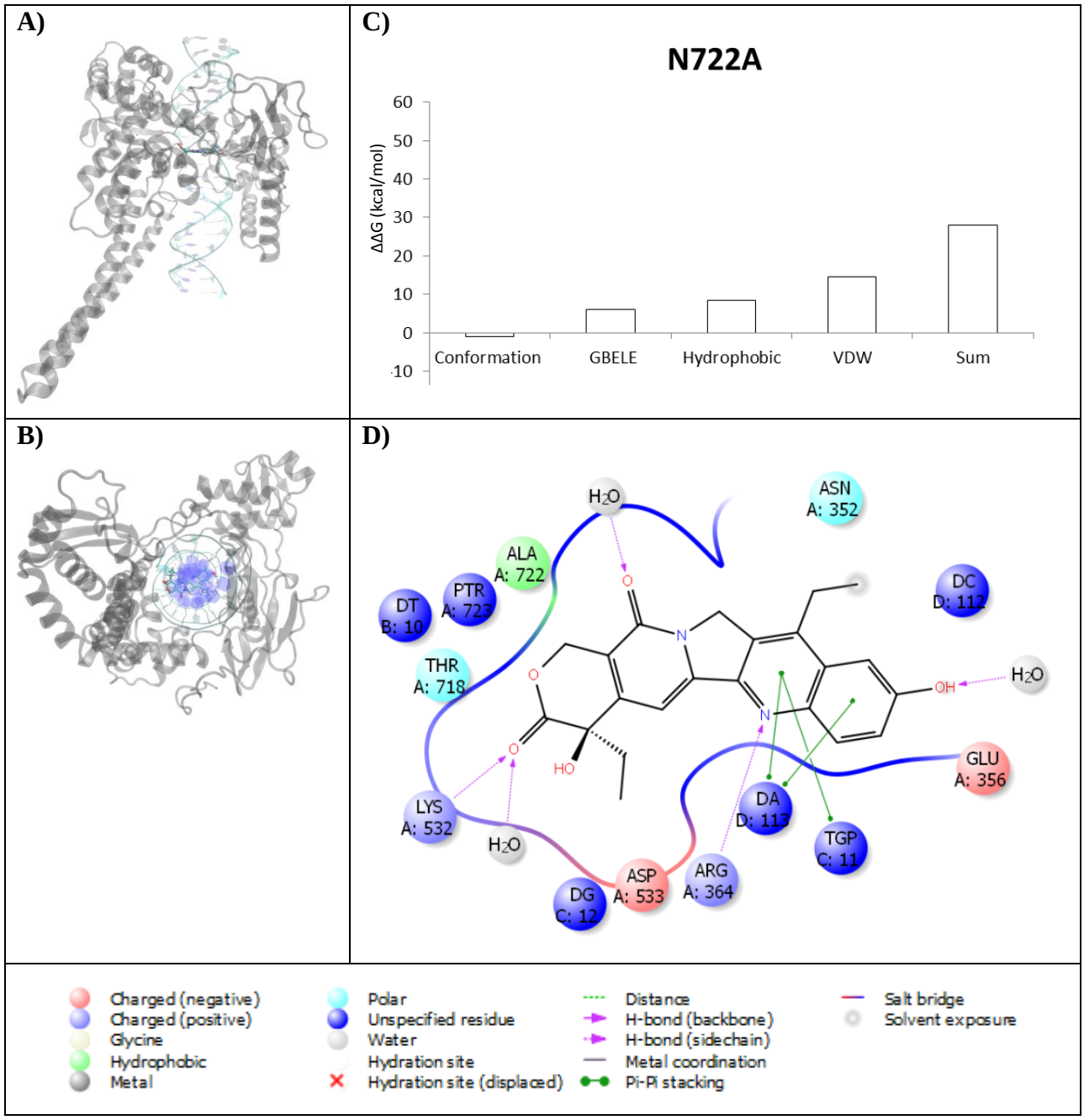


Figure **S25**. SN38 Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

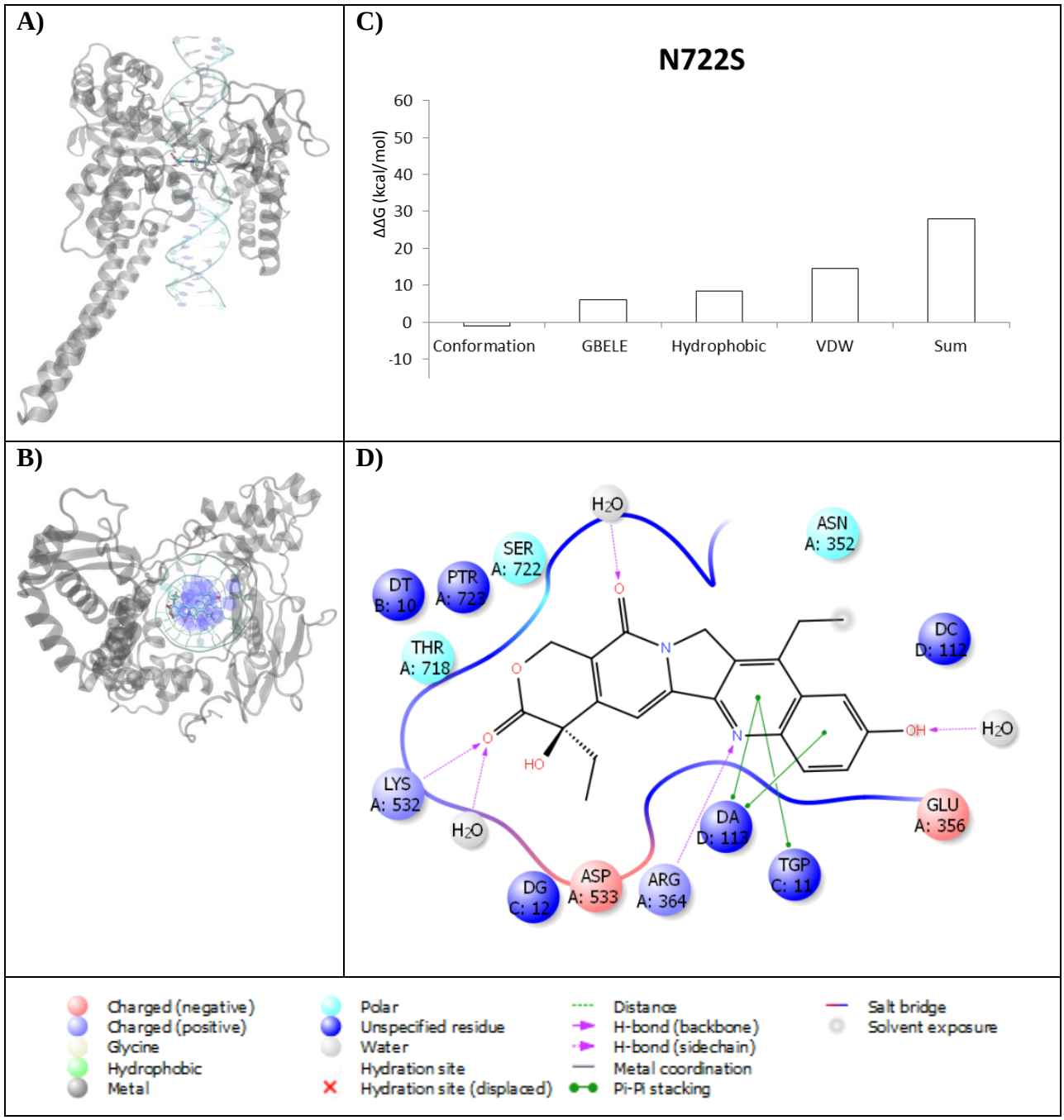


Figure **S26**. SN38 Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

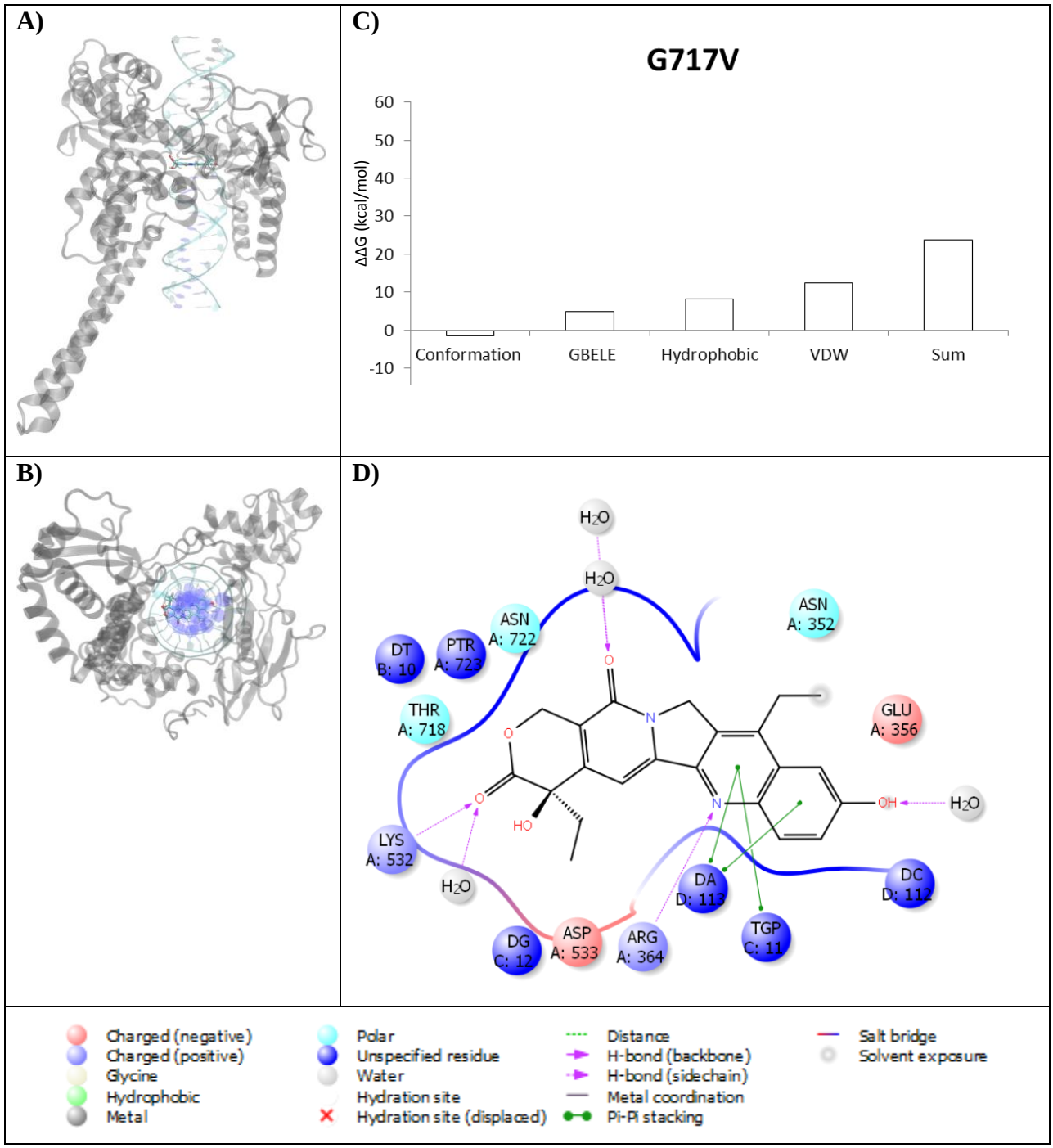


Figure **S27**. SN38 Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

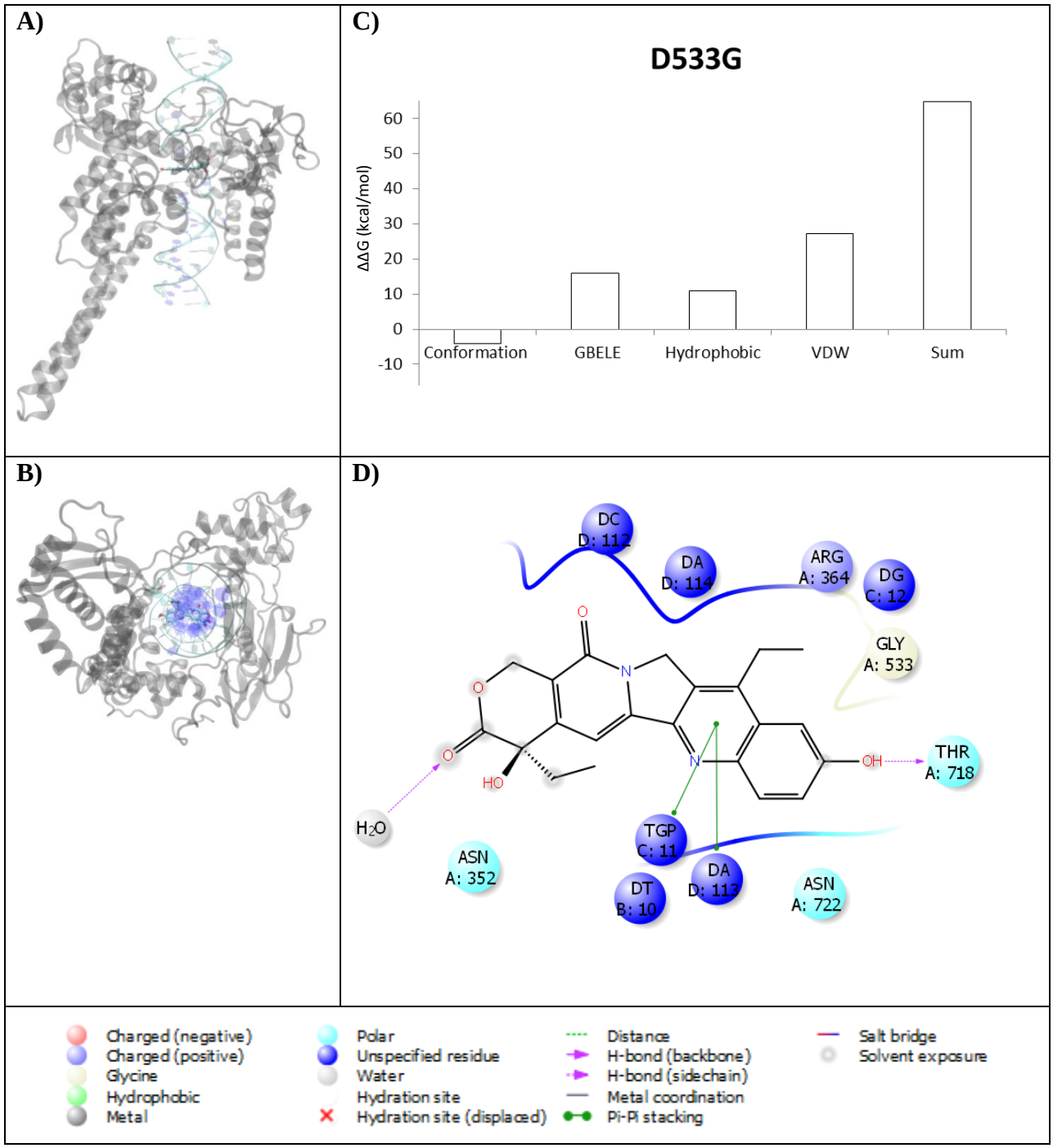


Figure **S28**. SN38 Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

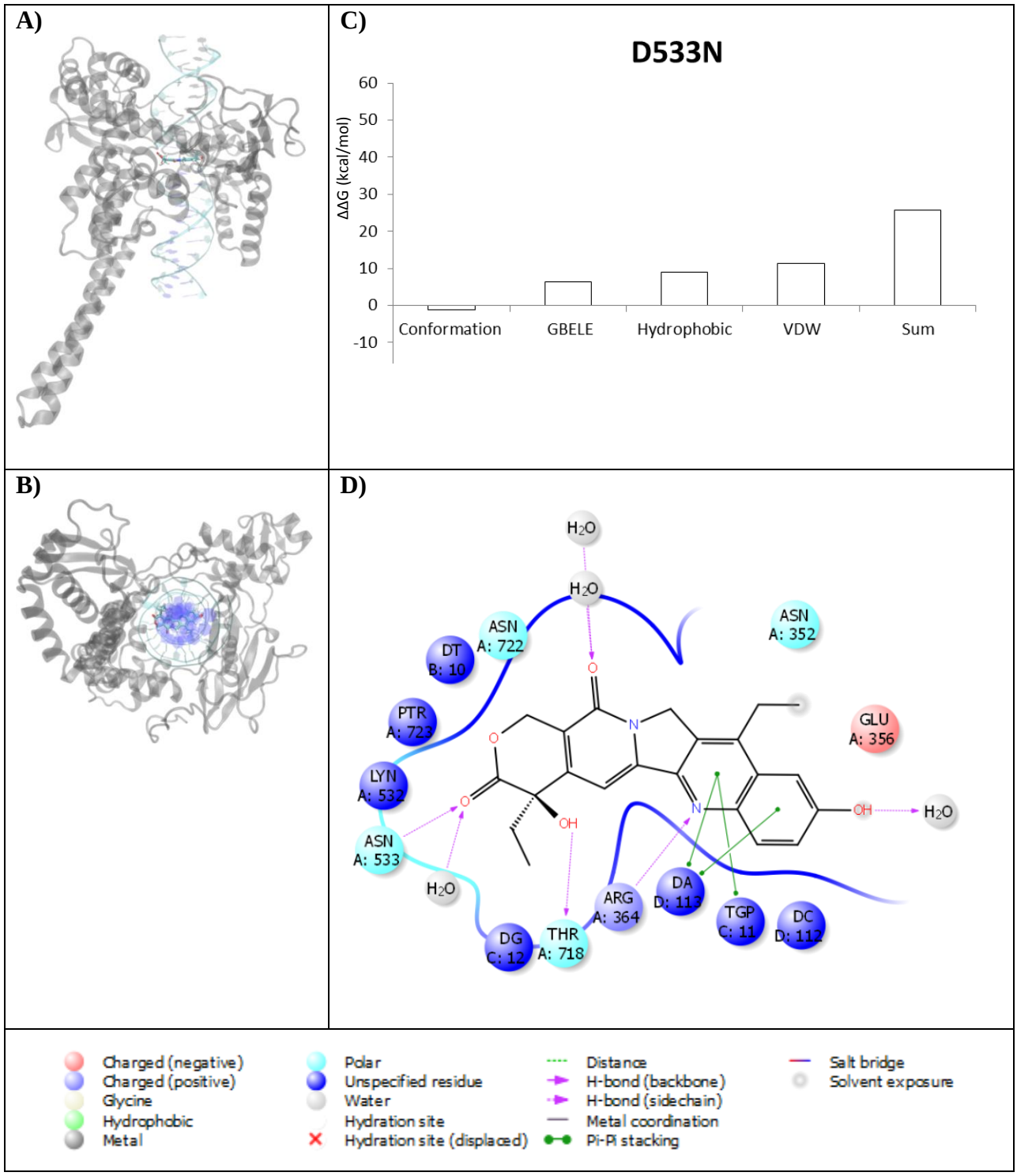


Figure **S29**. SN38 Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

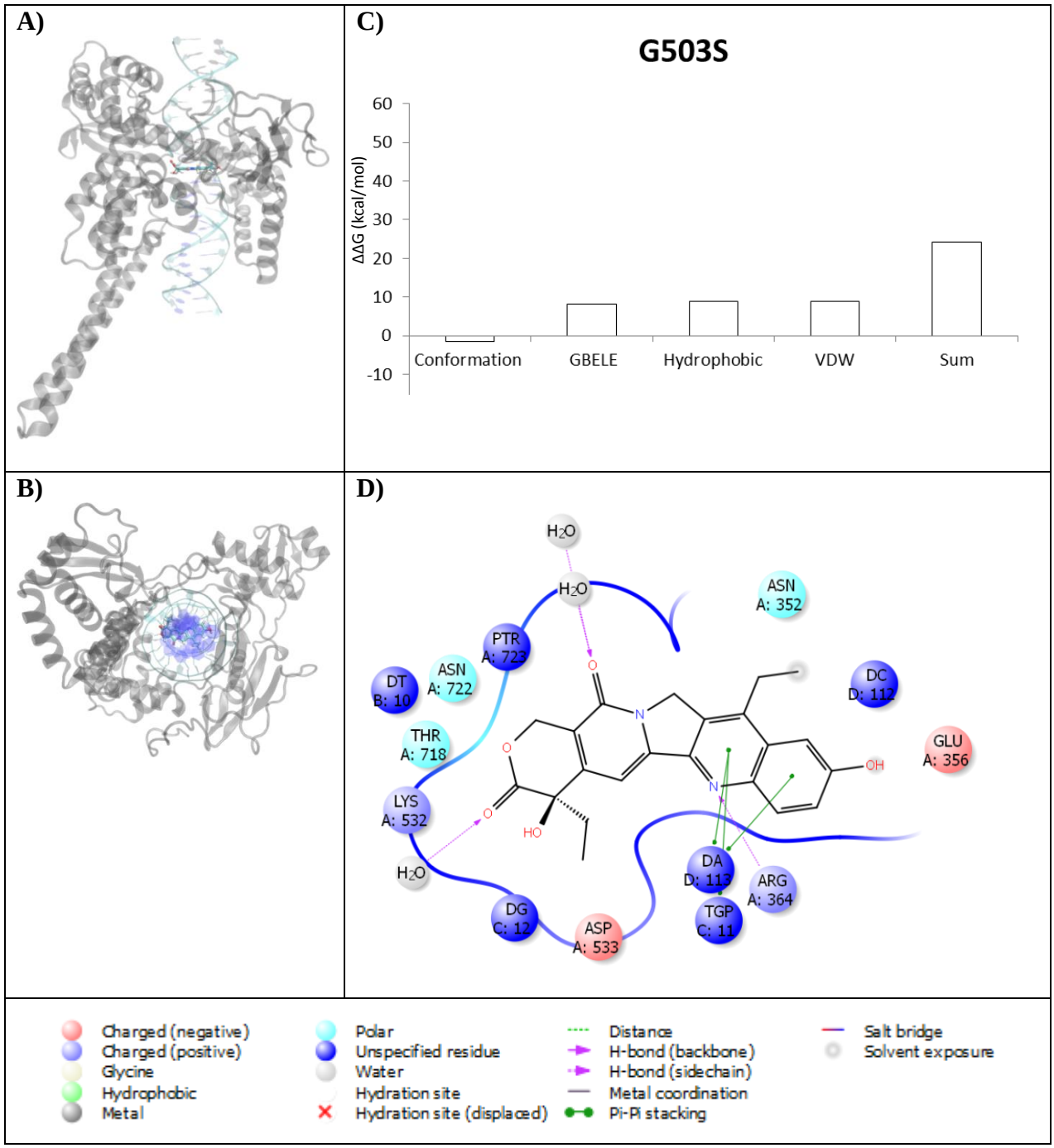


Figure **S30**. SN38 Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

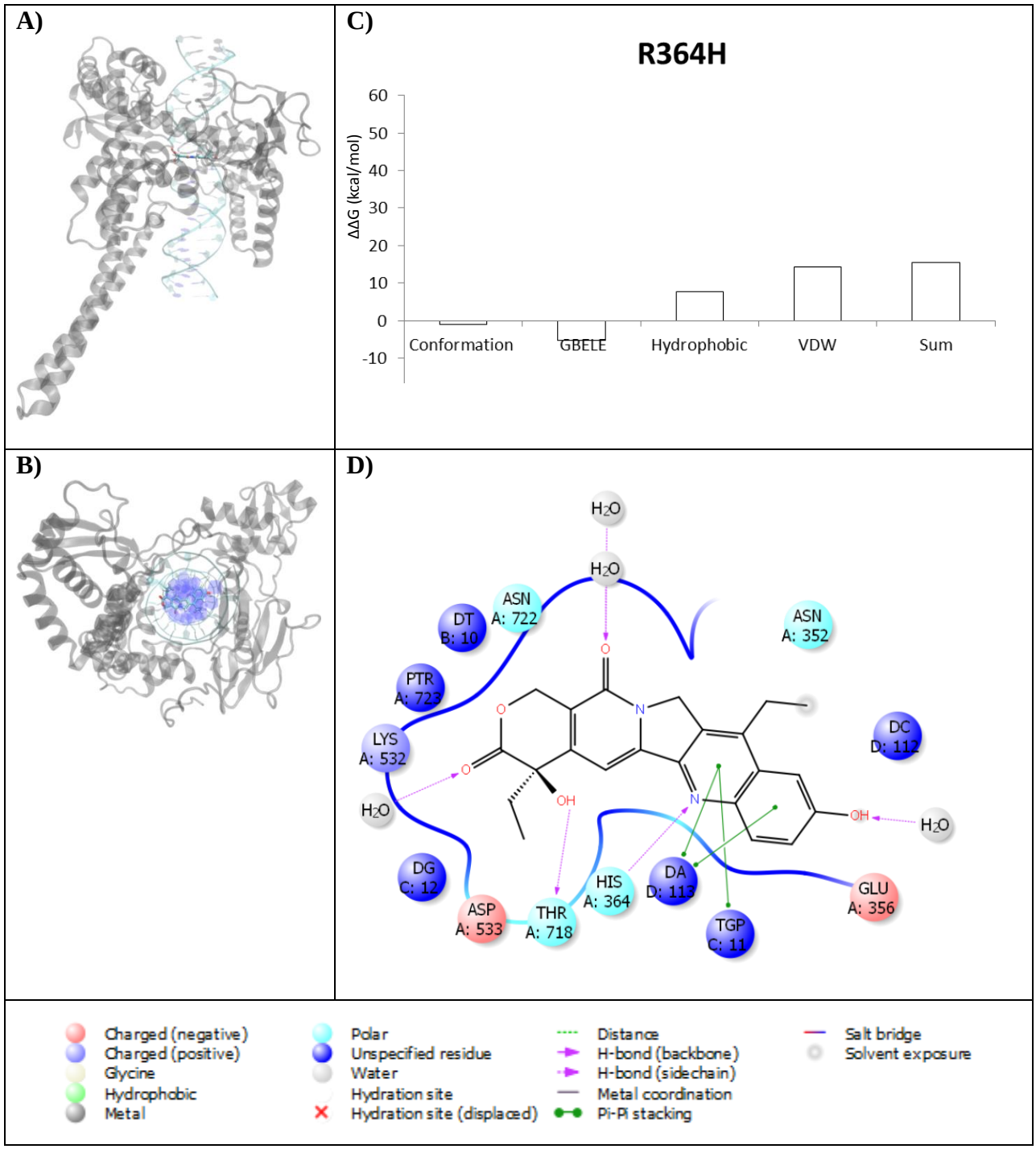


Figure **S31**. SN38 Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

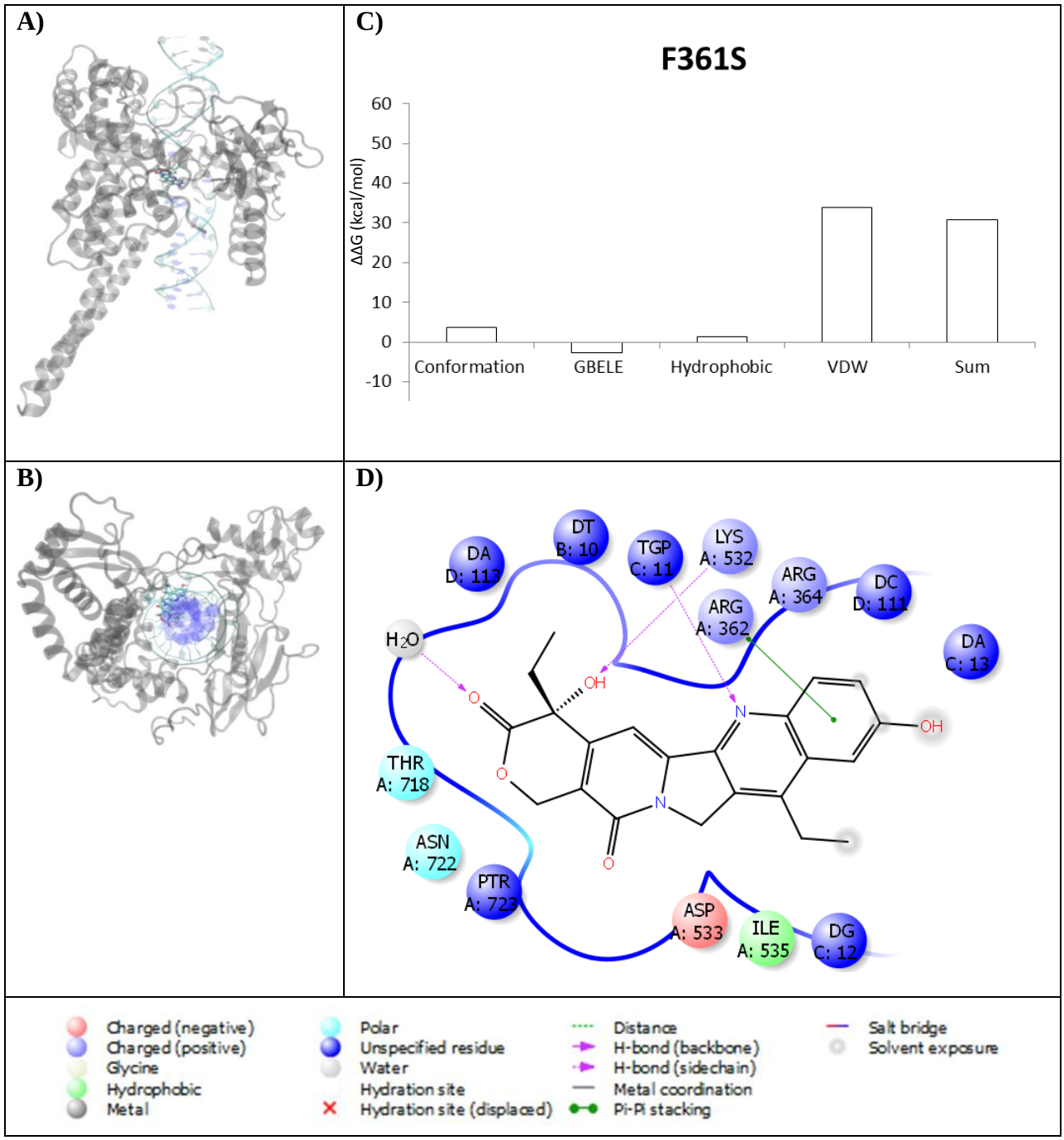


Figure **S32**. SN38 Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

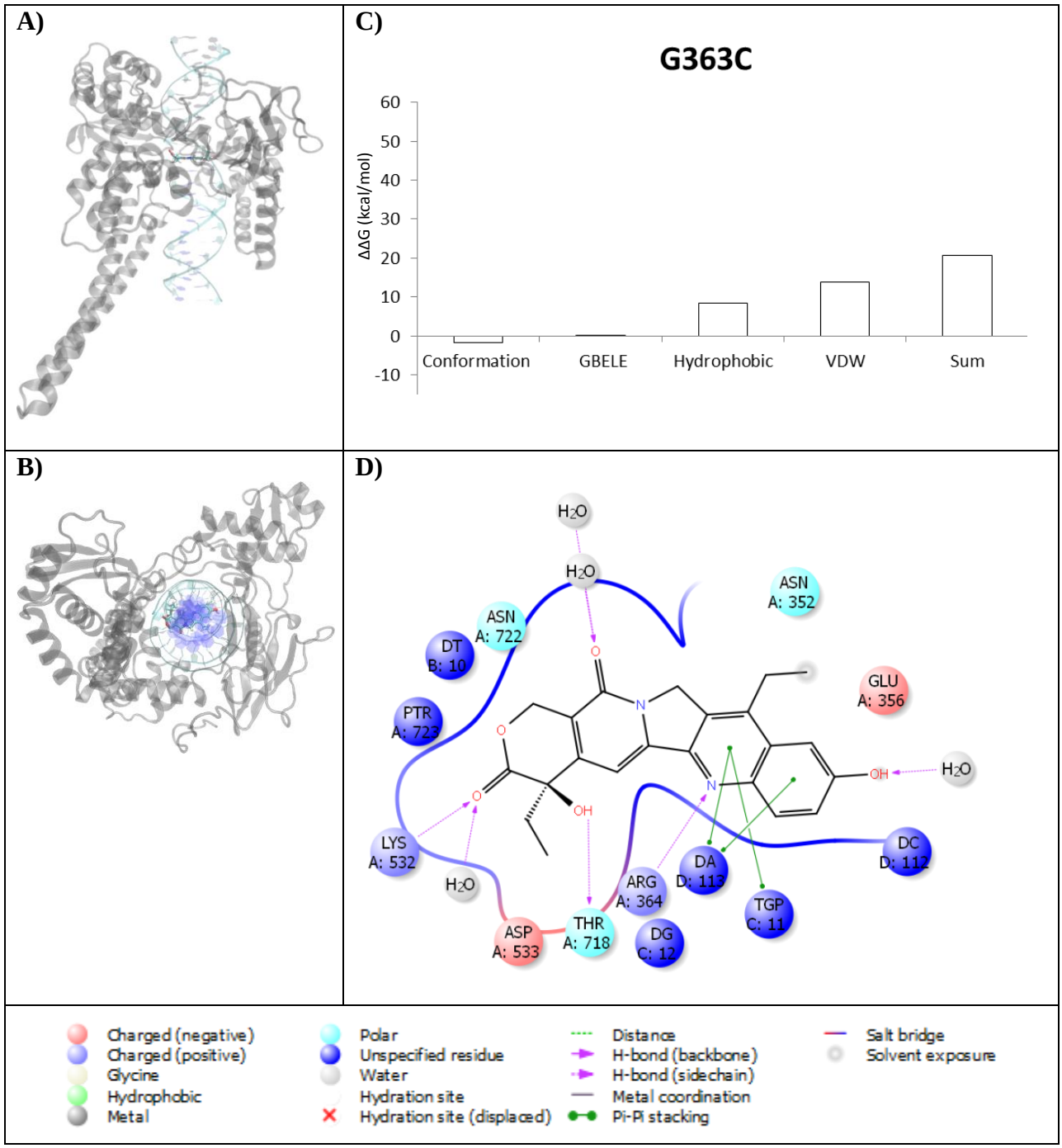


Figure **S33**. SN38 Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

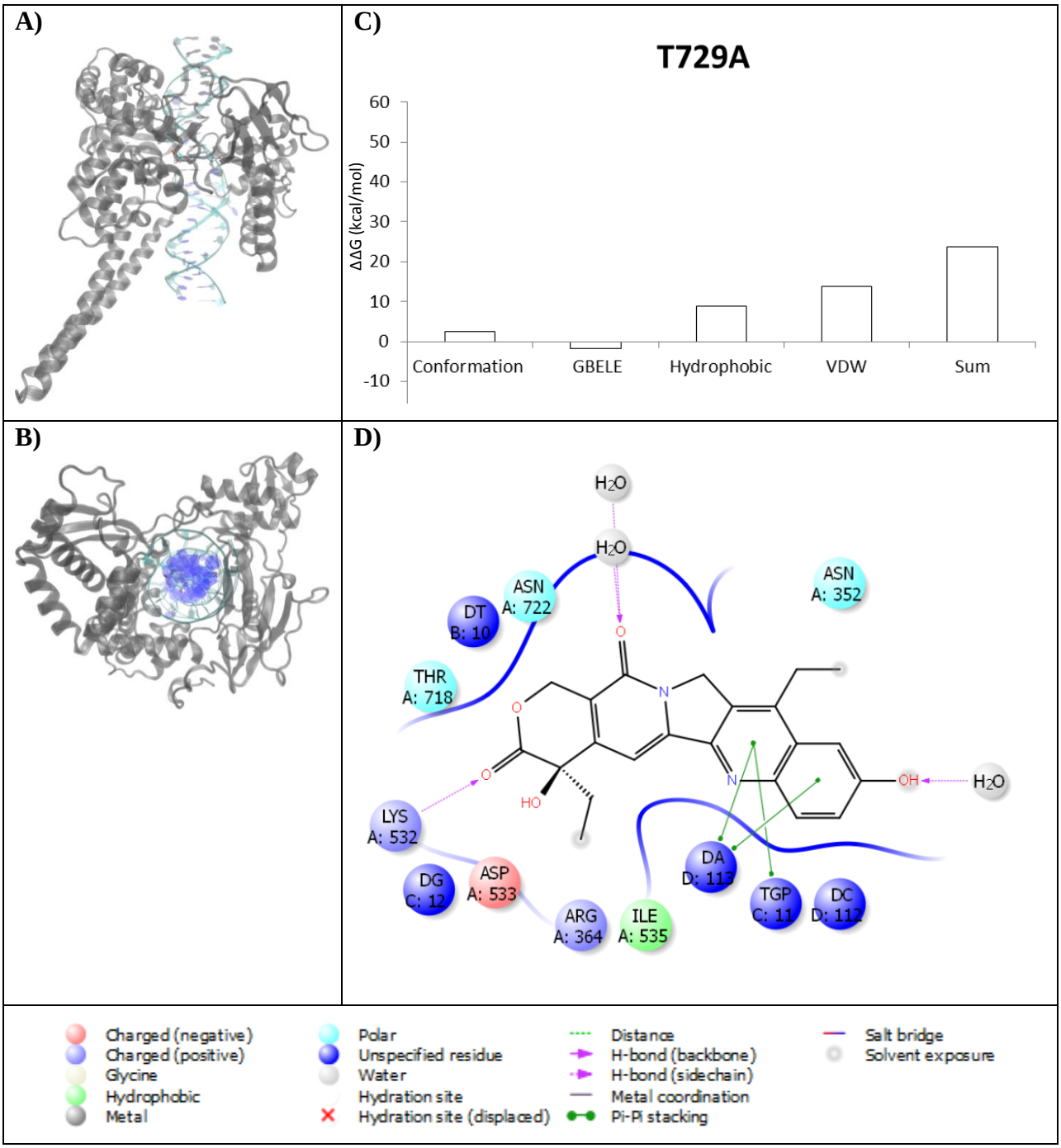


Figure **S34**. SN38 Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

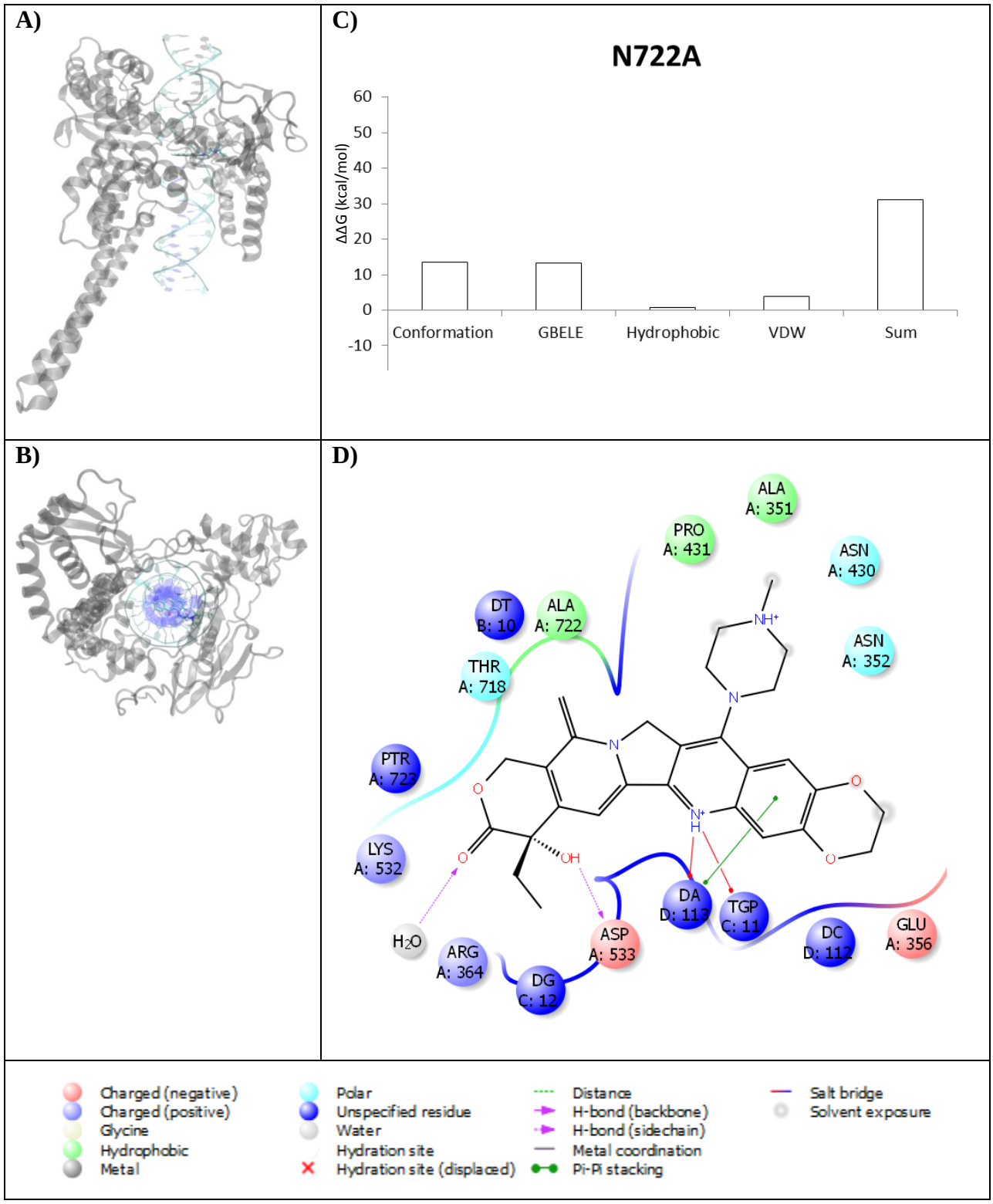


Figure **S35**. Lurtotecan Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

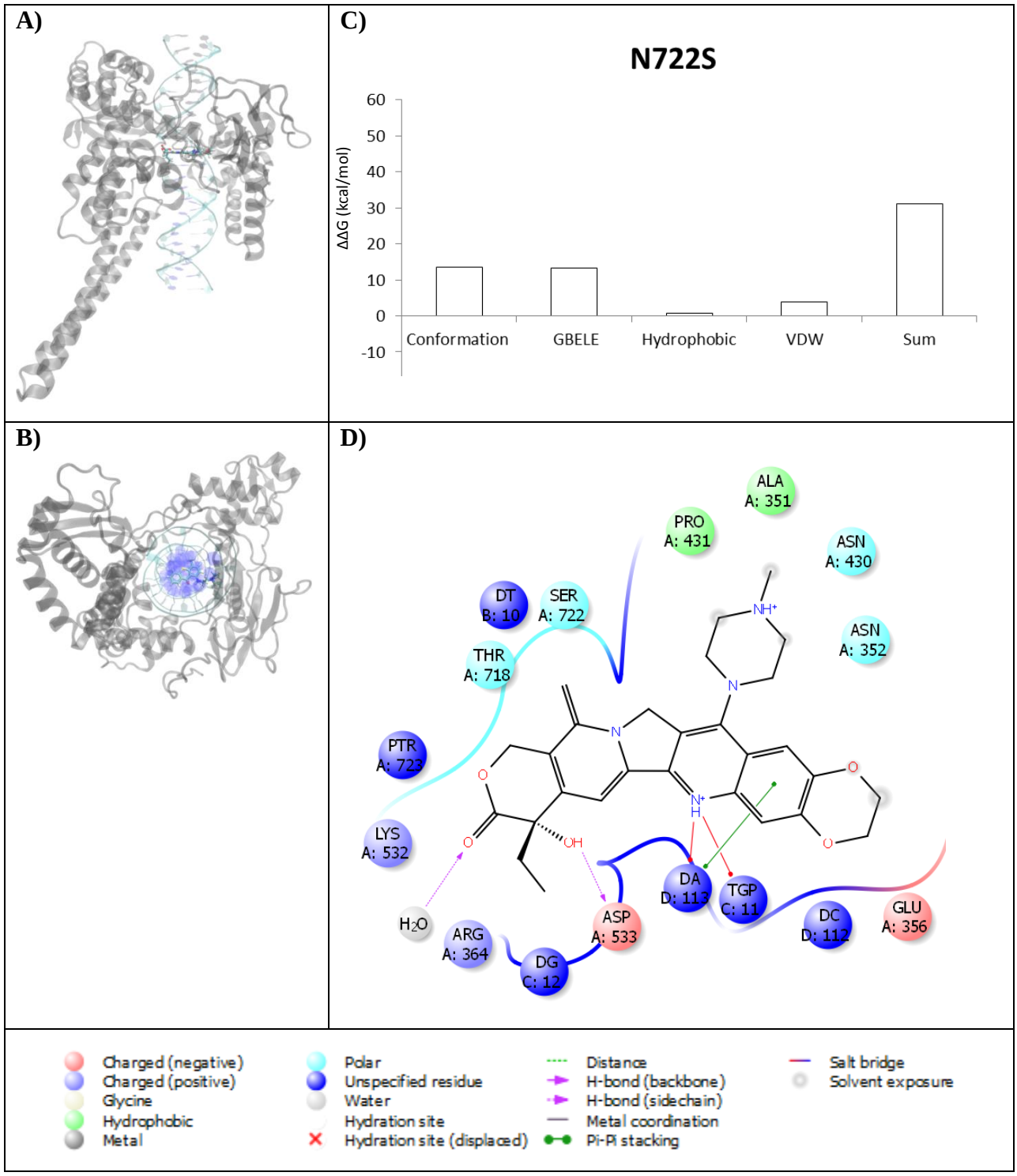


Figure **S36**. Lurtotecan Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

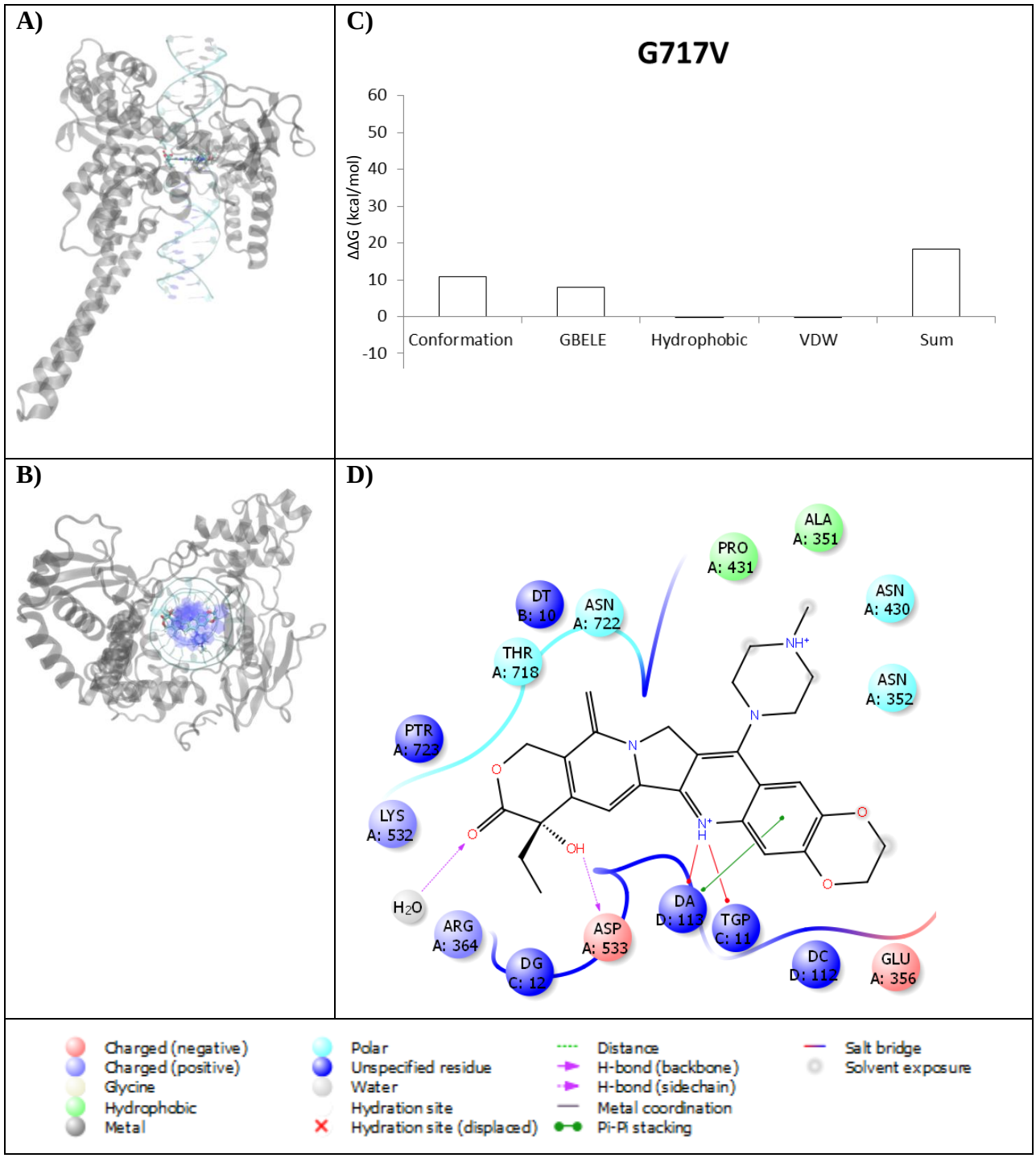


Figure **S37**. Lurtotecan Bound to Complex. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

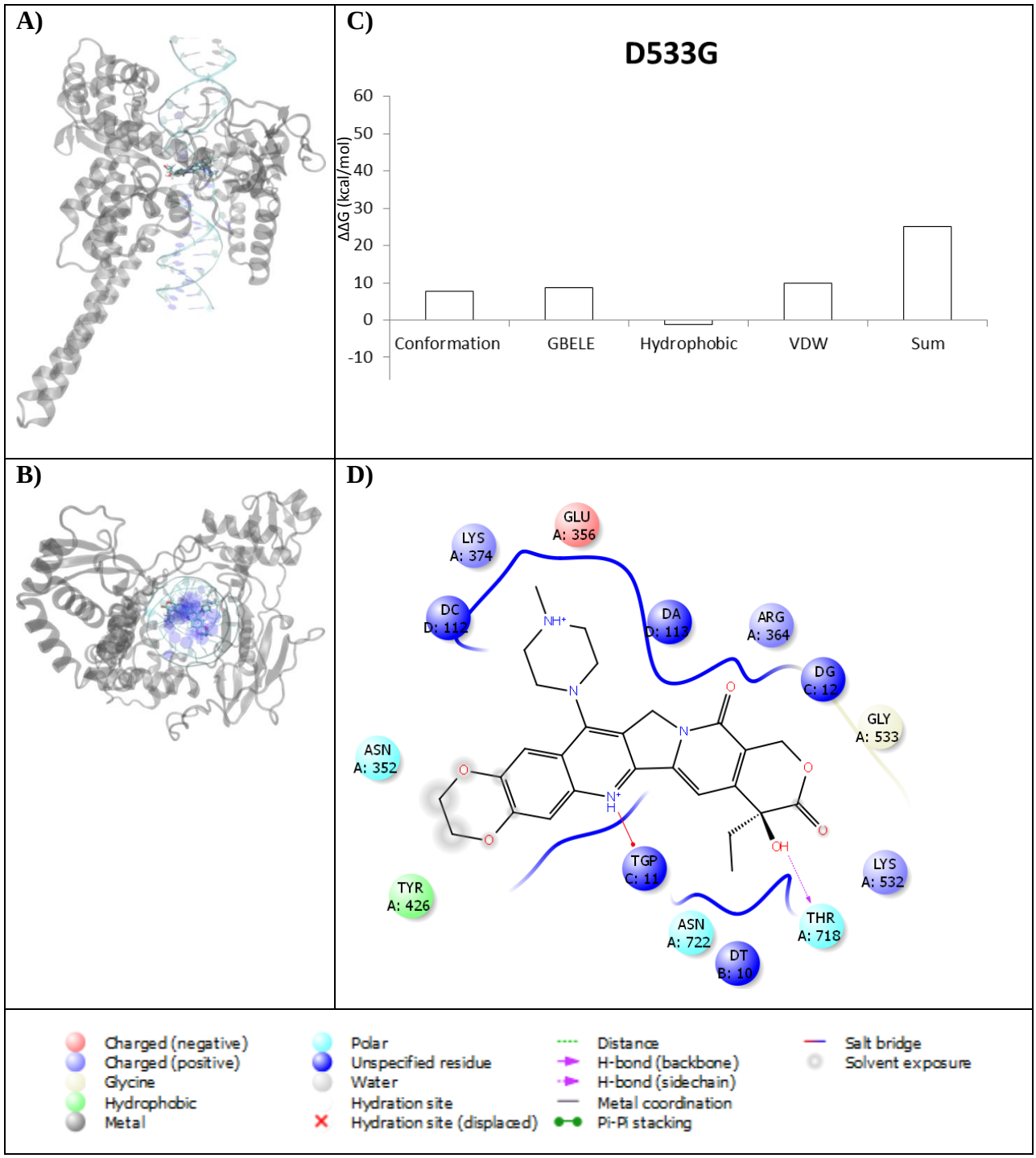


Figure **S38**. Lurtotecan Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

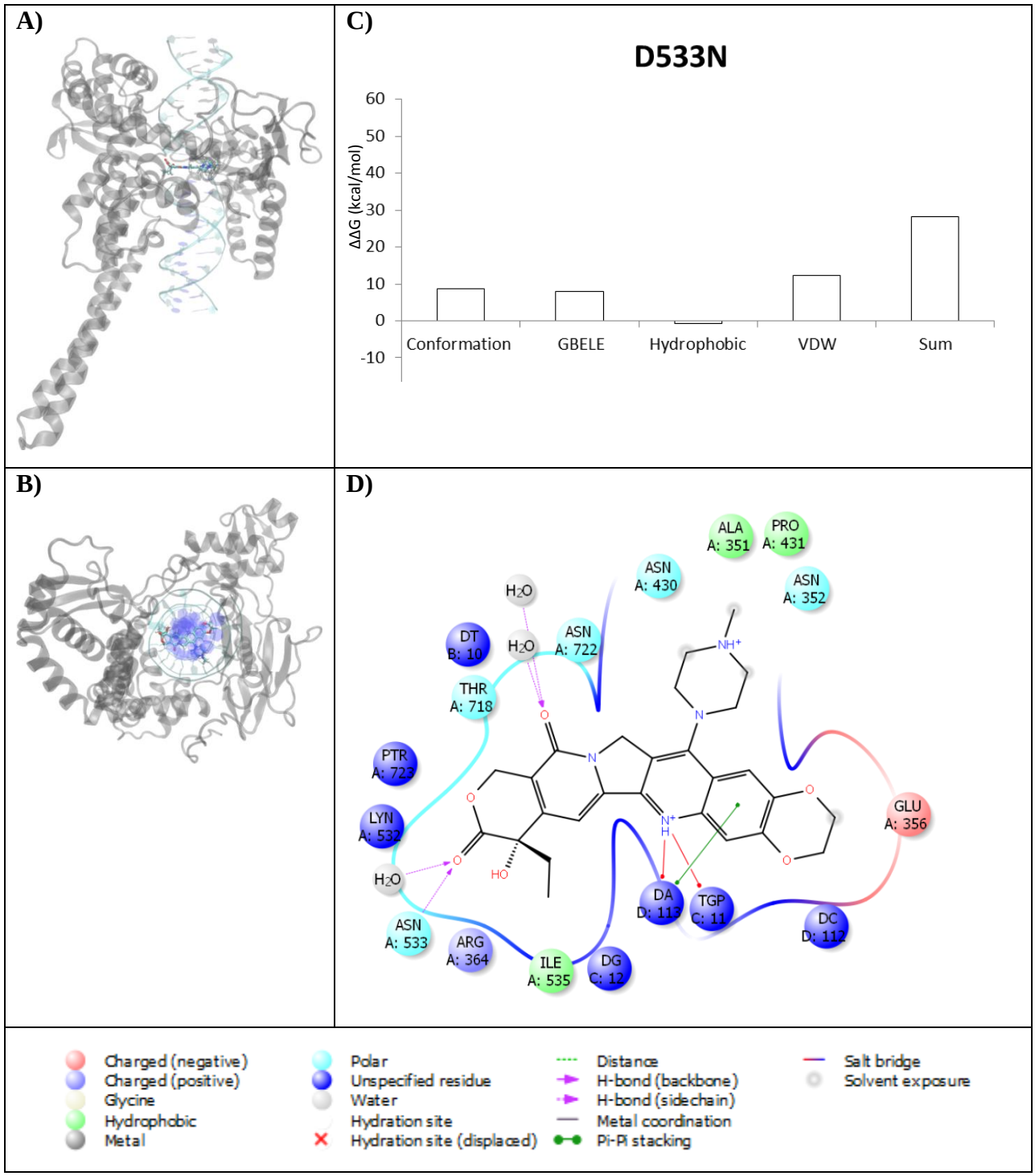


Figure **S39**. Lurtotecan Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

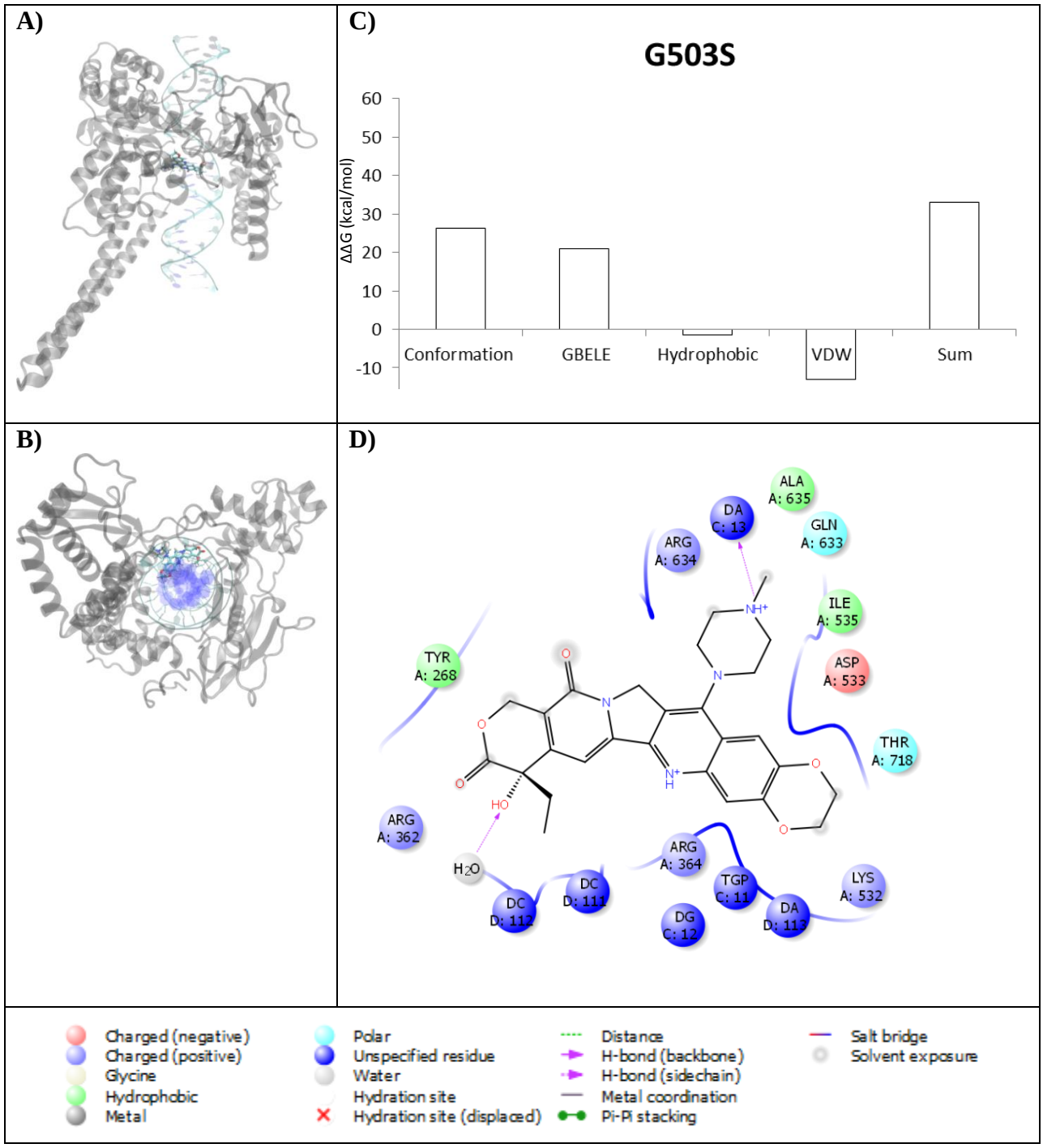


Figure **S40**. Lurtotecan Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

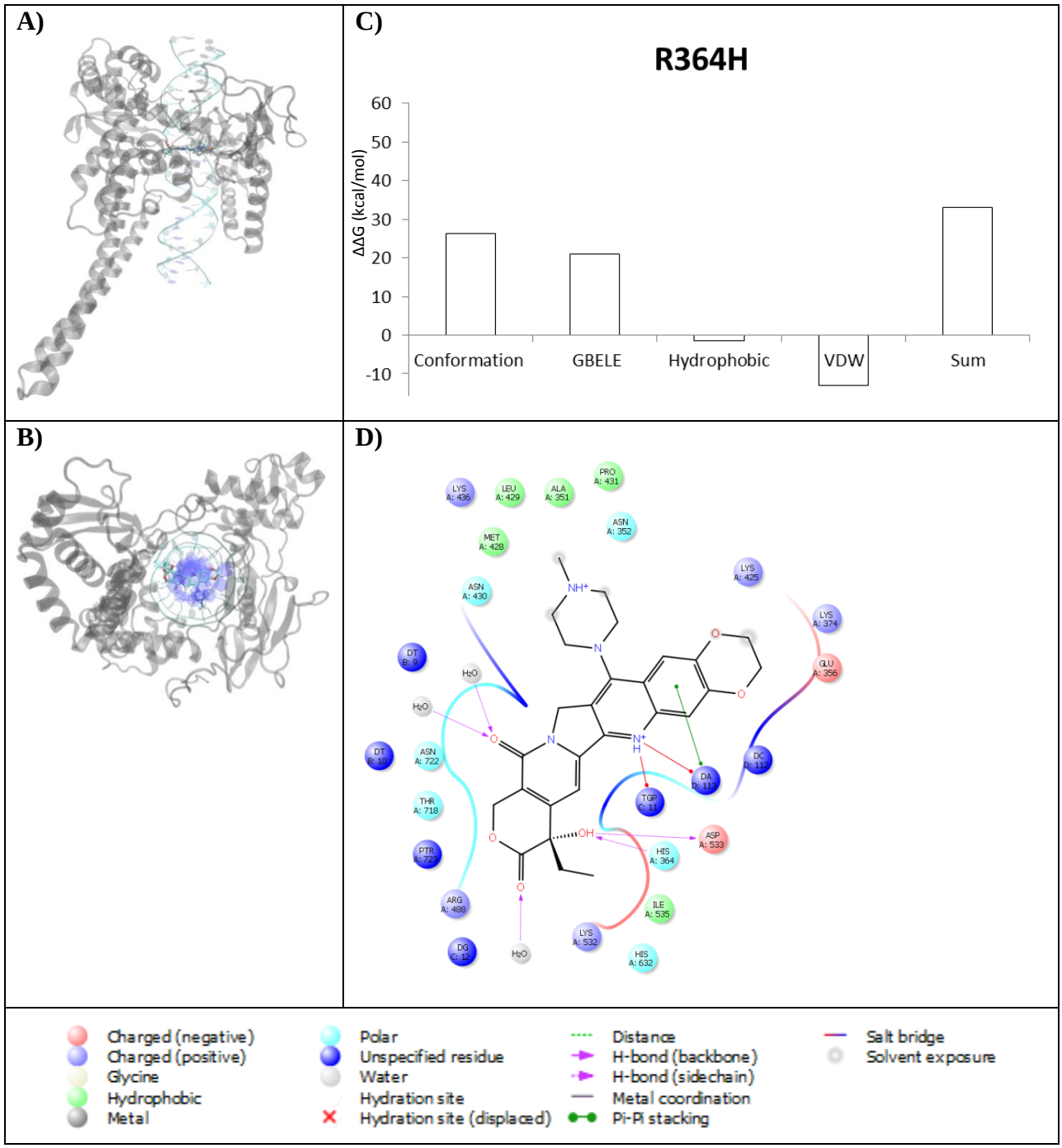


Figure **S41**. Lurtotecan Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

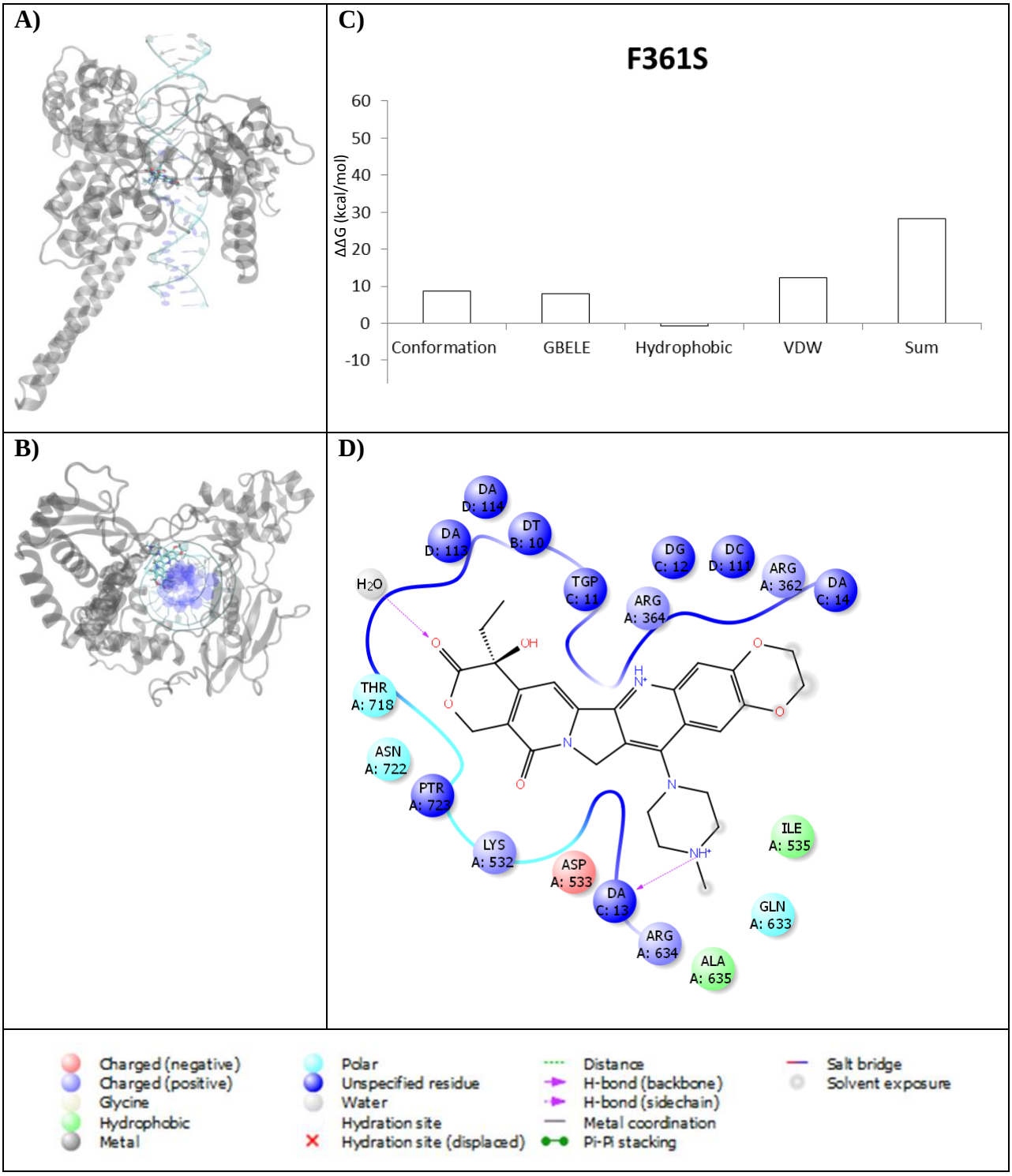


Figure **S42**. Lurtotecan Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

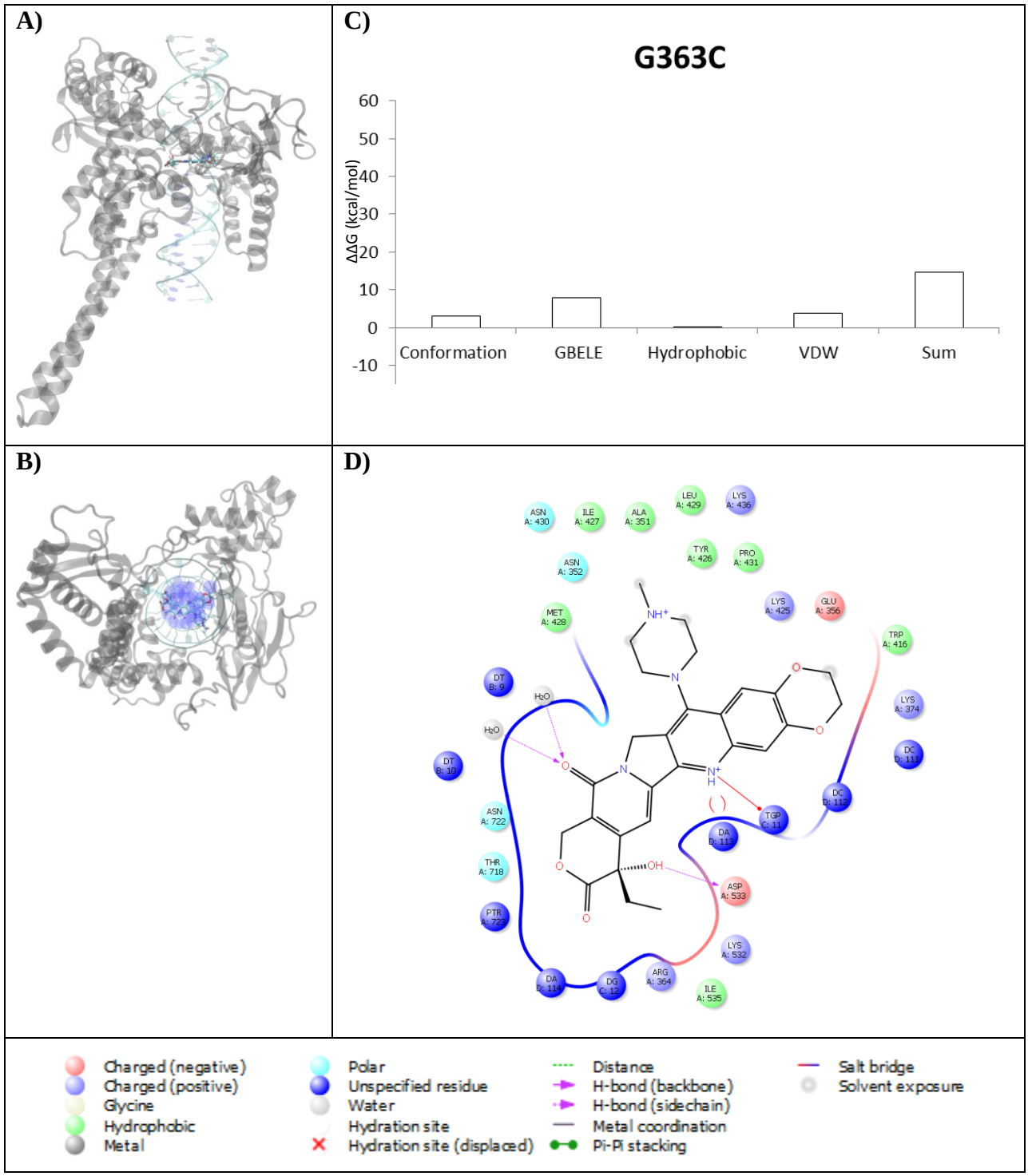


Figure **S43**. Lurtotecan Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

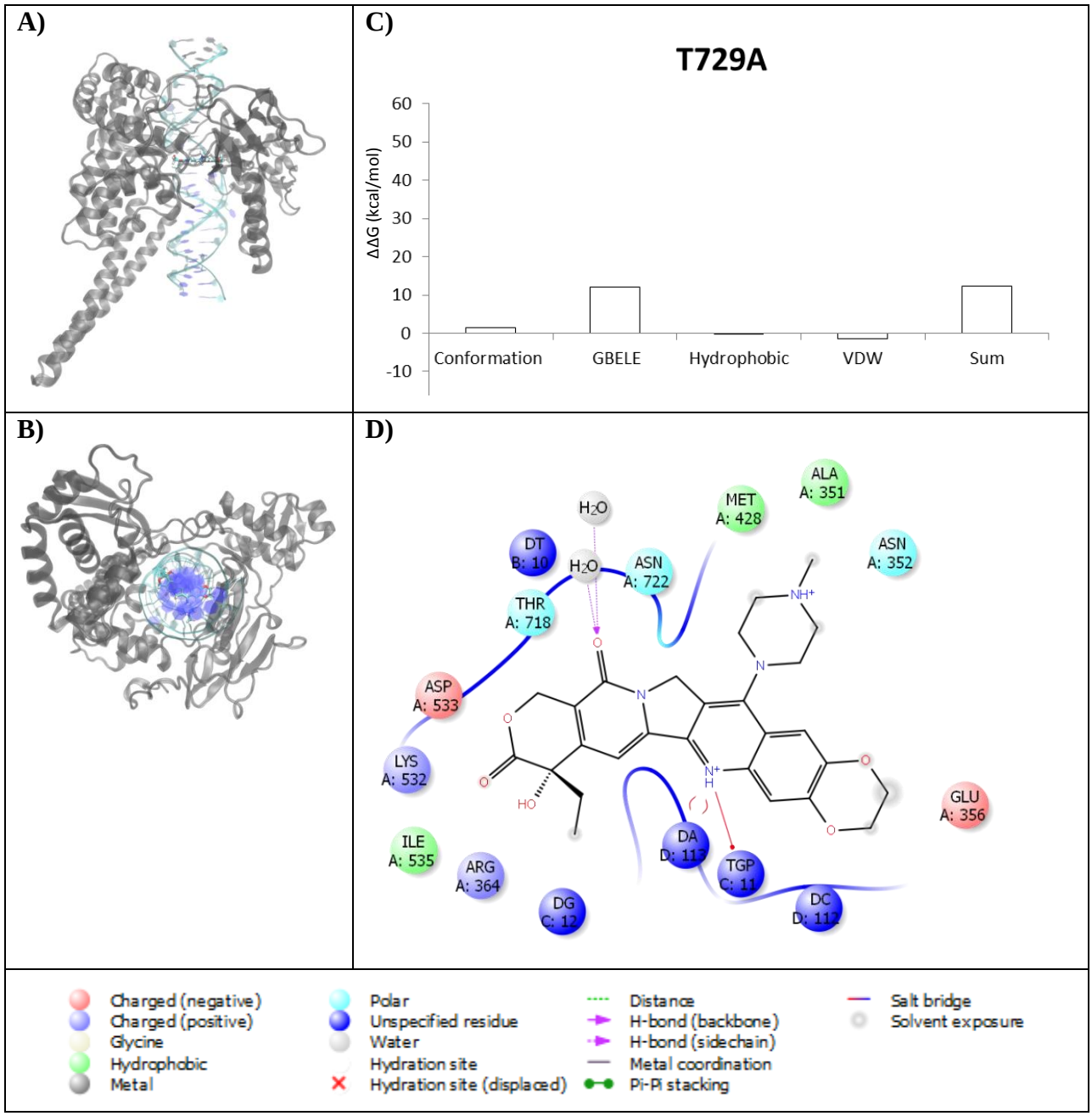


Figure **S44**. Lurtotecan Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

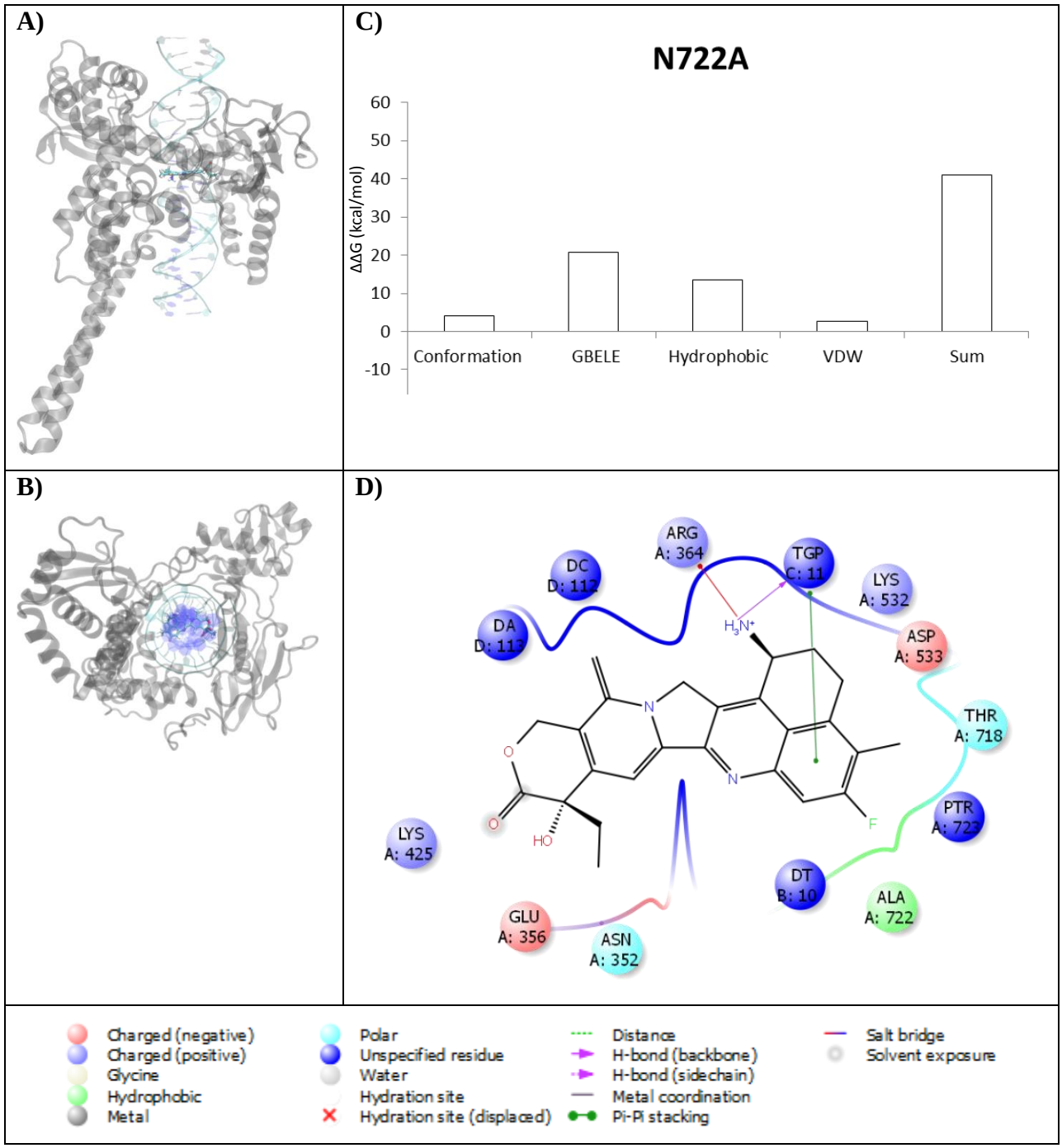


Figure **S45**. Exatecan Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

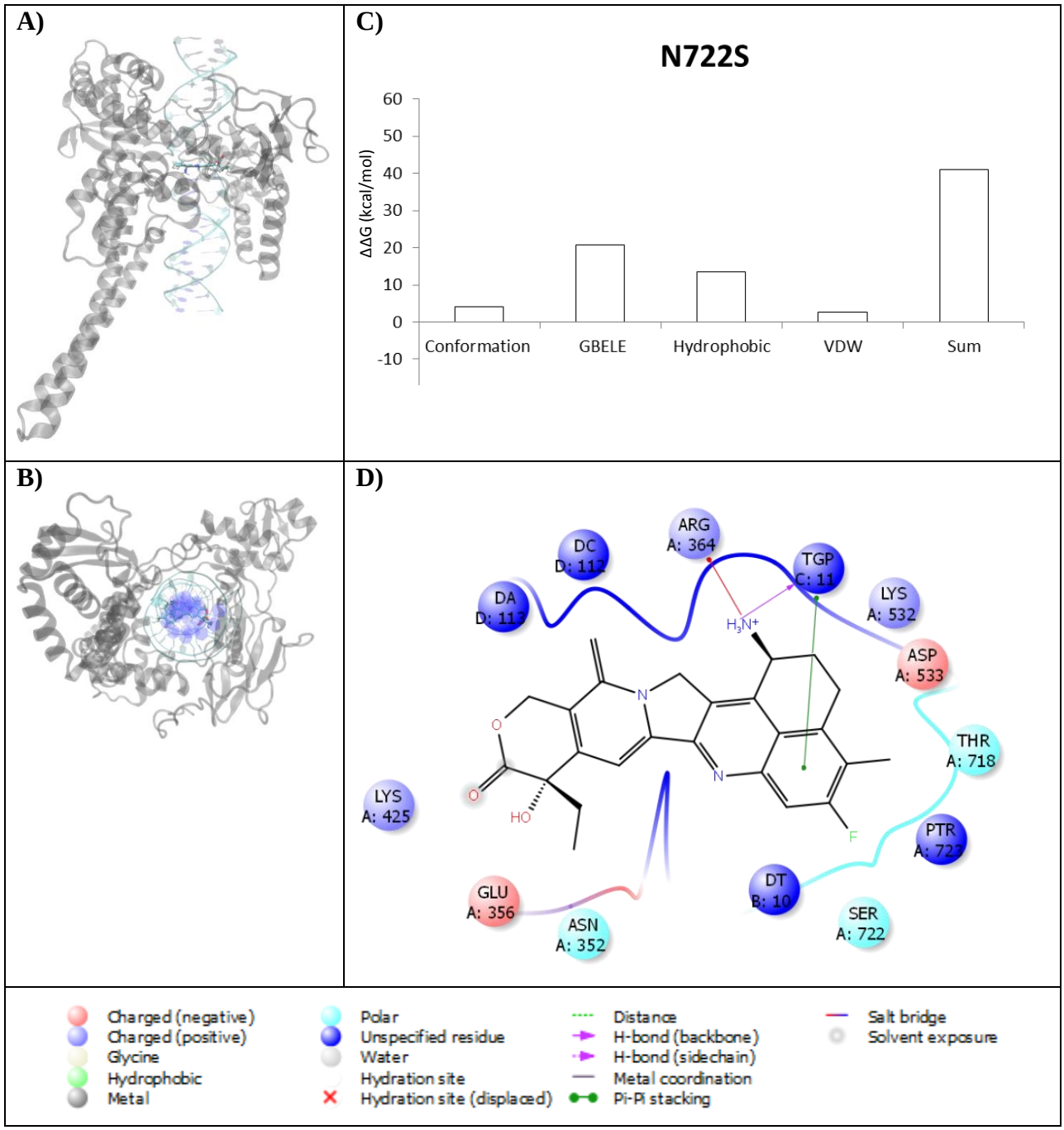


Figure **S46**. Exatecan Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

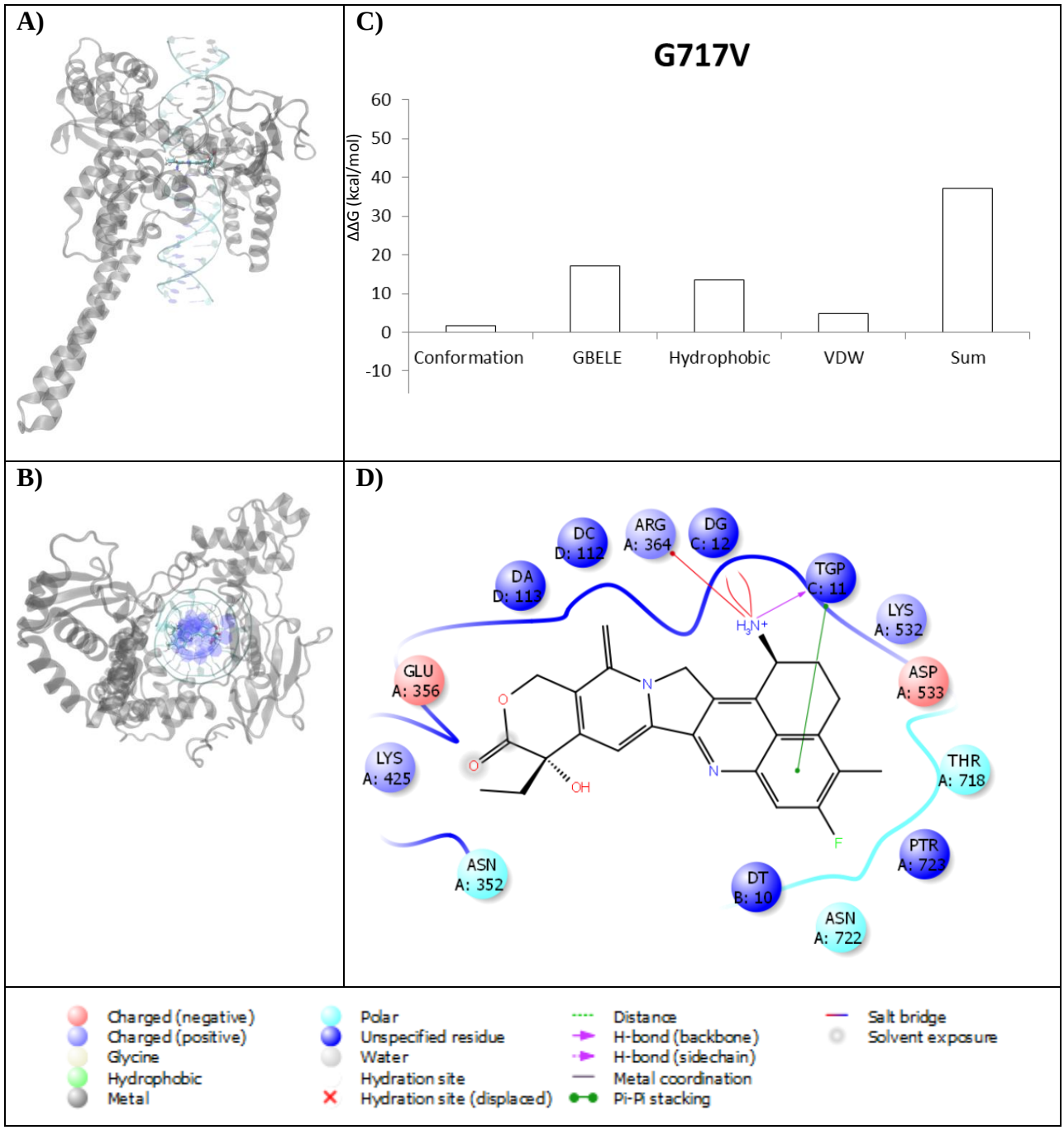


Figure **S47**. Exatecan Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

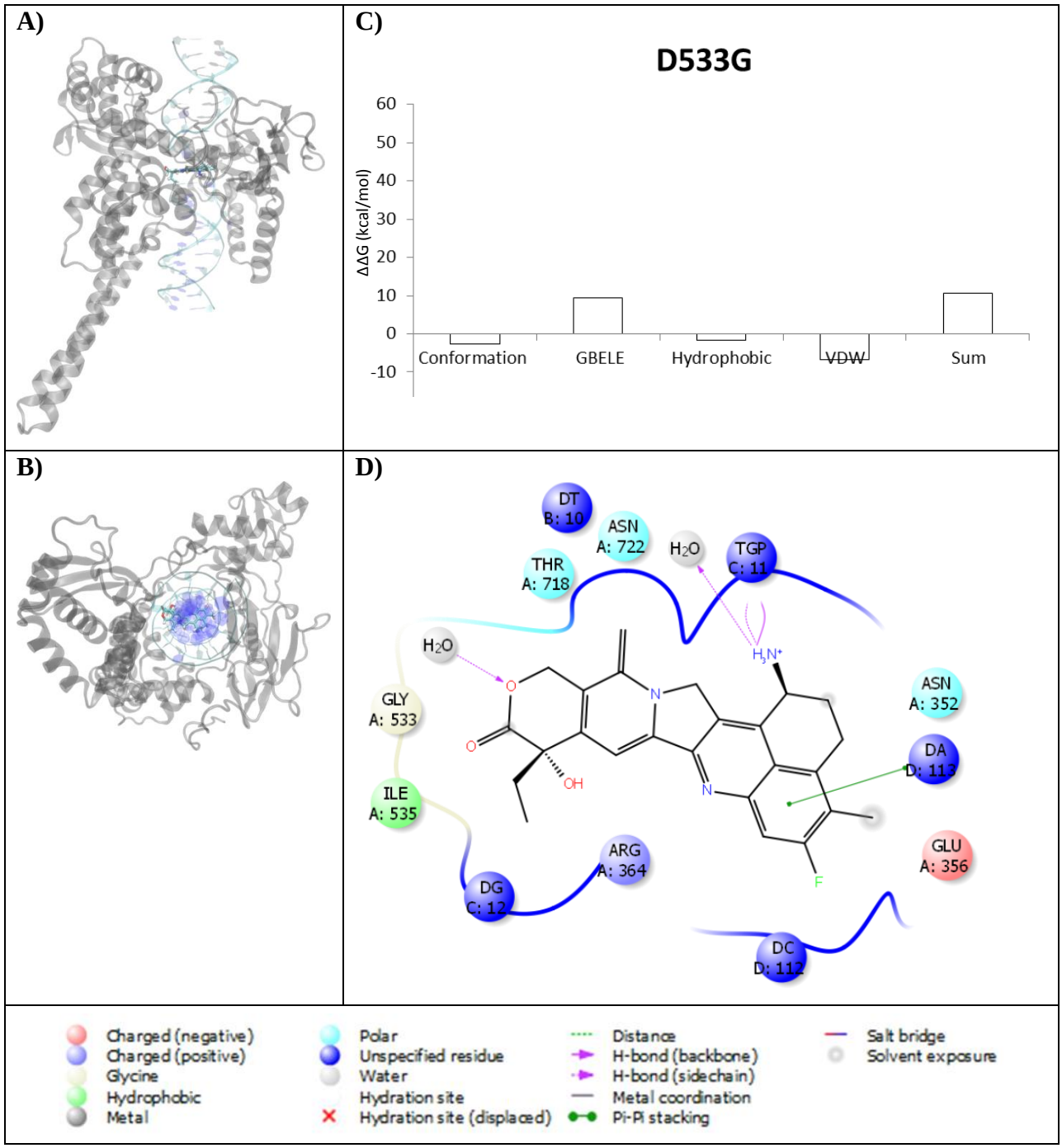


Figure **S48**. Exatecan Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

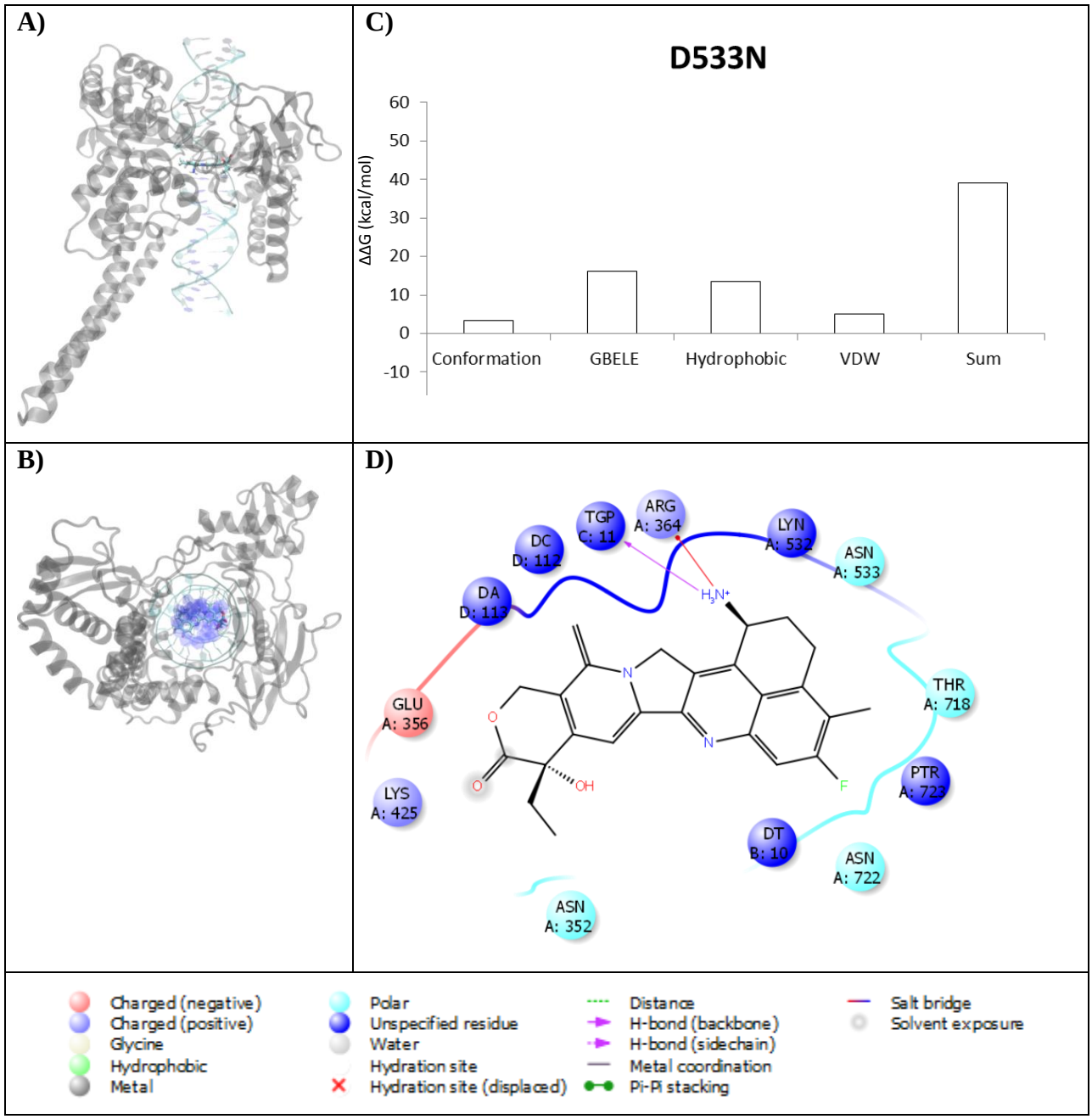


Figure **S49**. Exatecan Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

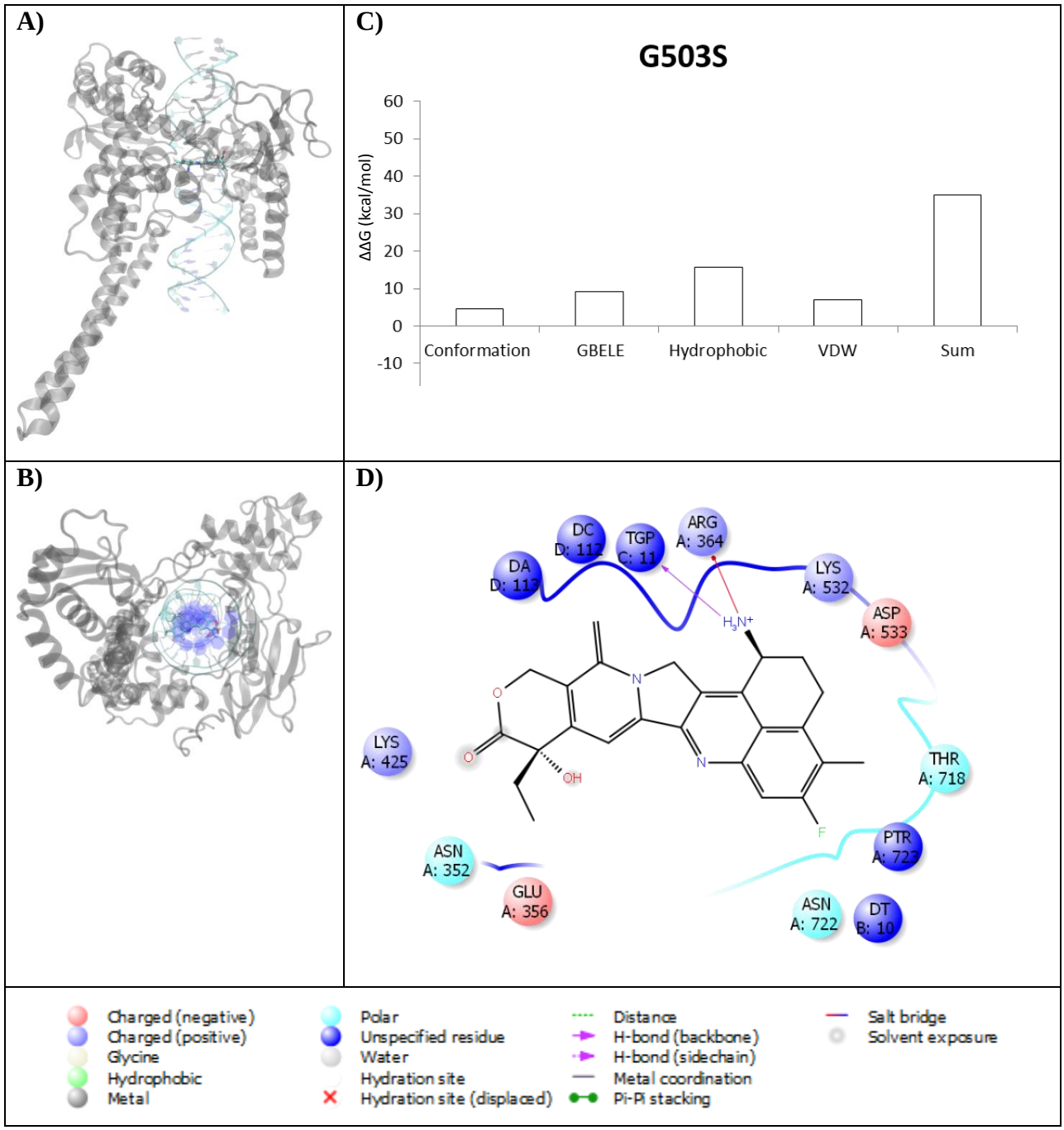


Figure **S50**. Exatecan Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

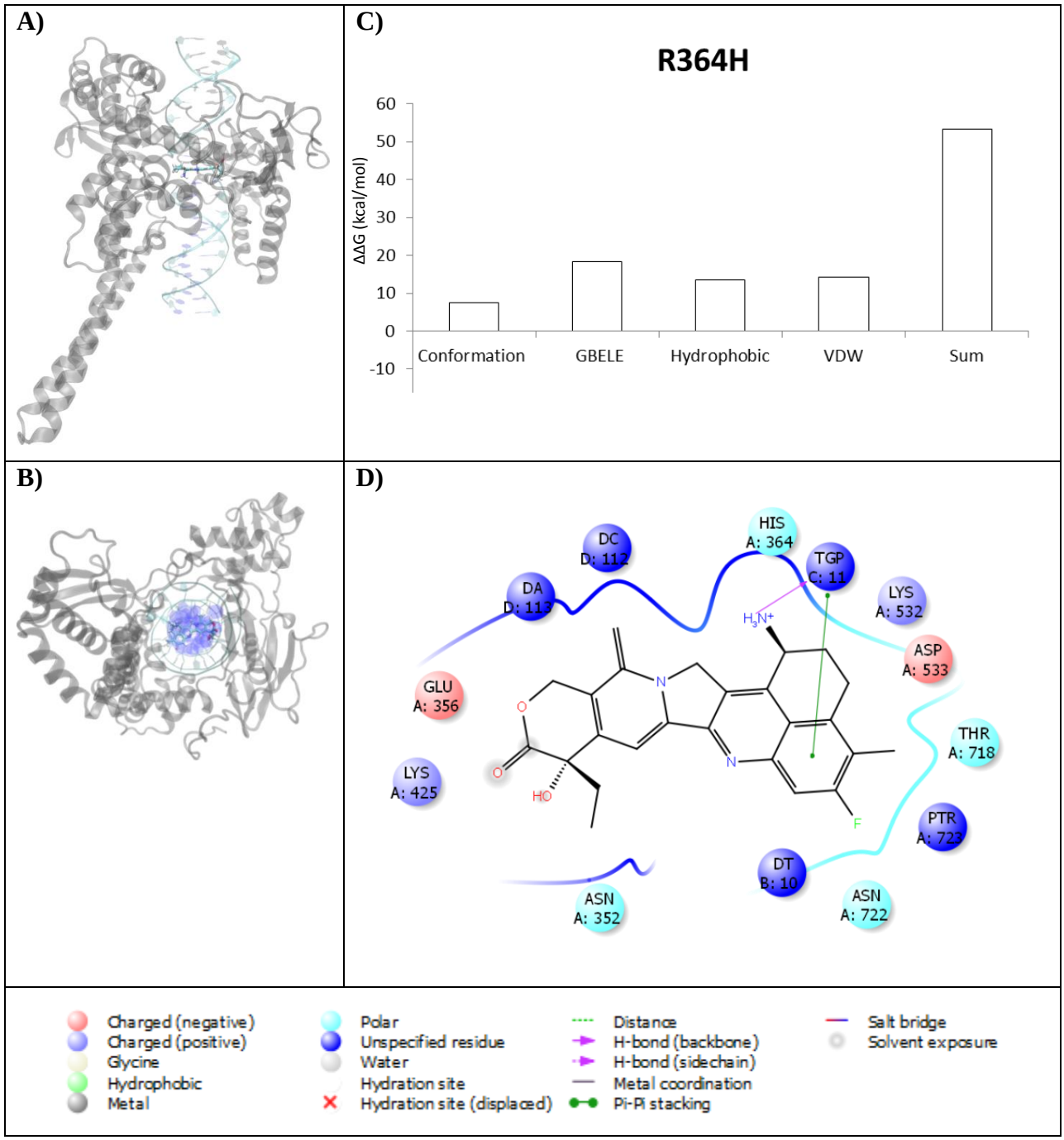


Figure **S51**. Exatecan Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

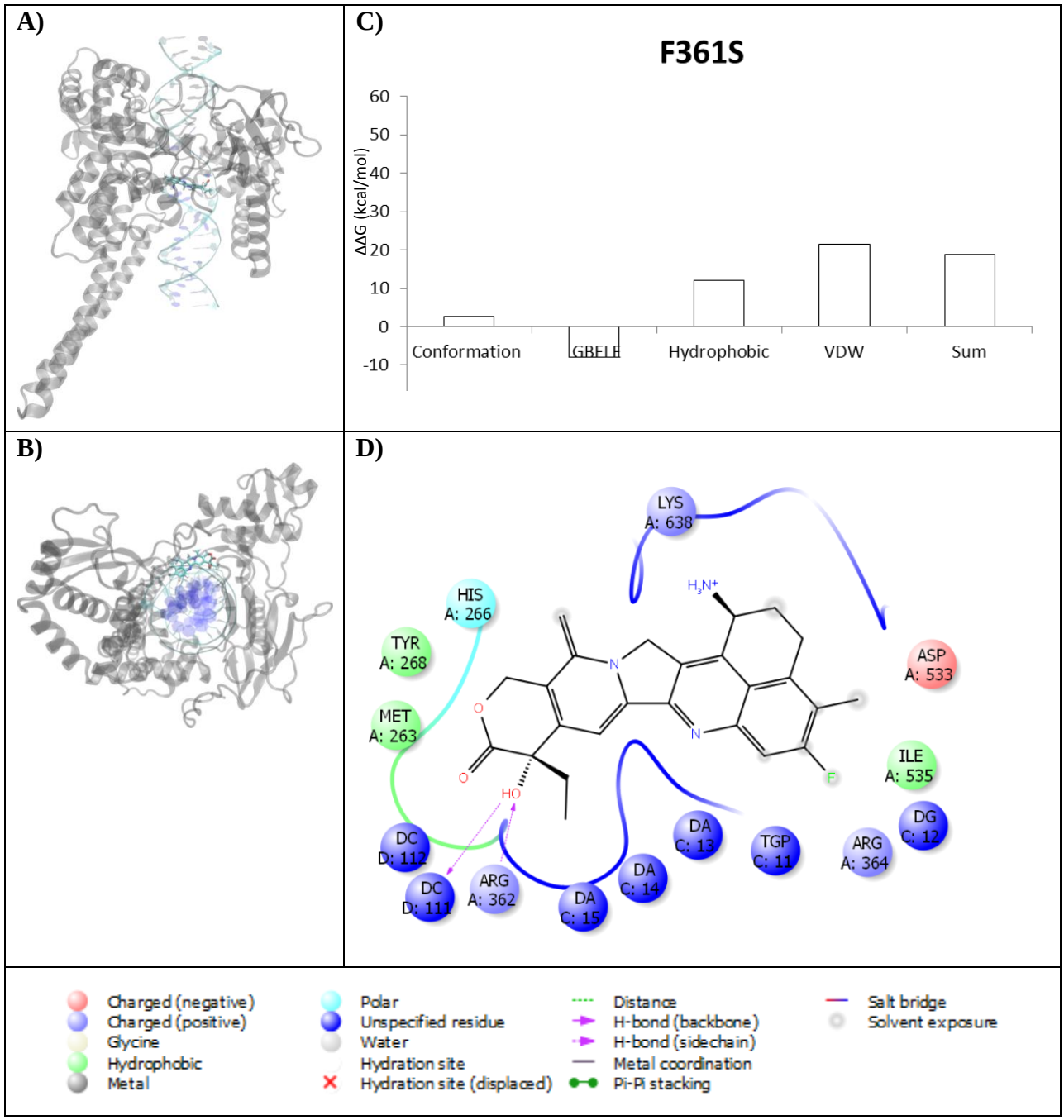


Figure **S52**. Exatecan Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

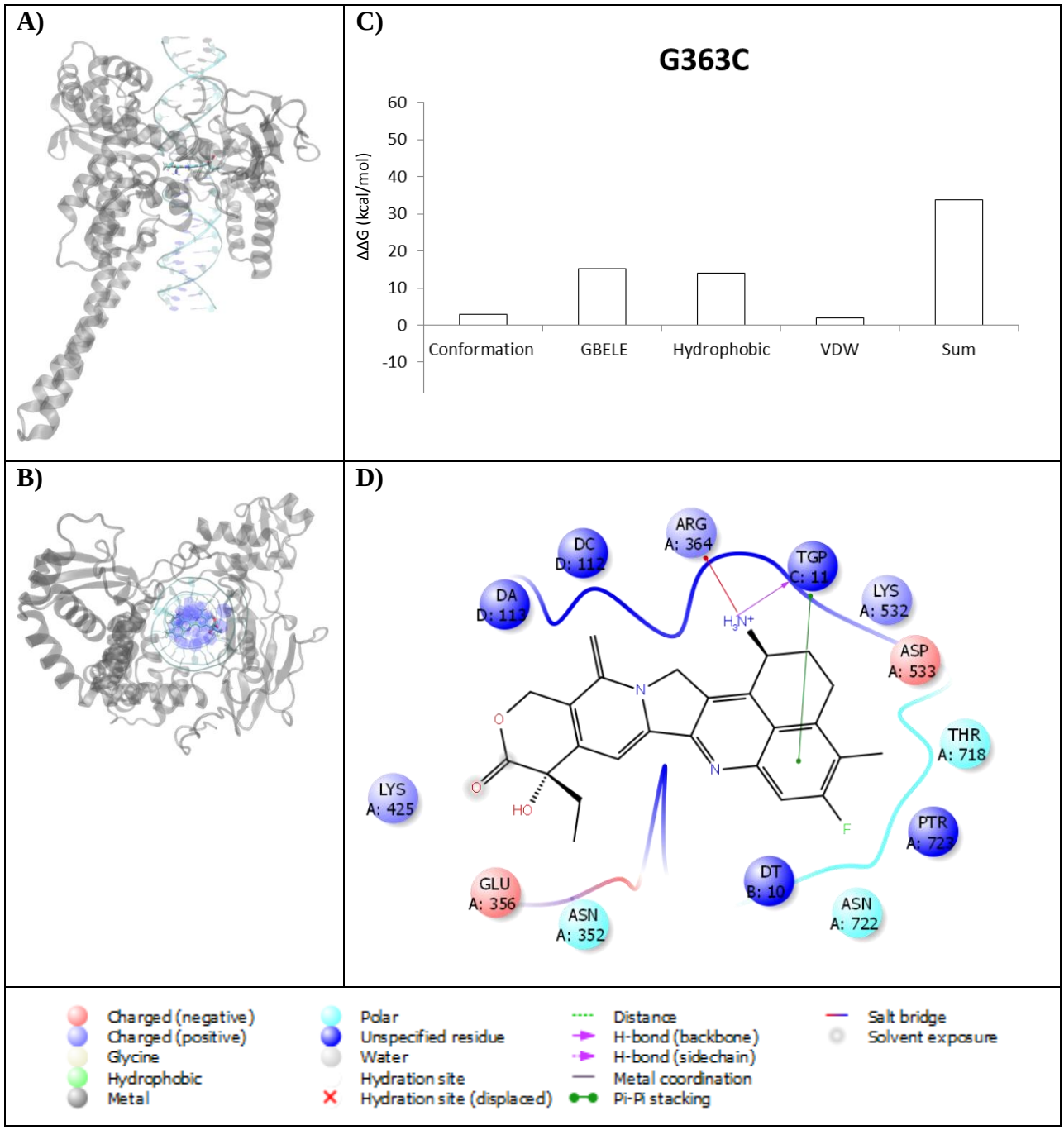


Figure **S53**. Exatecan Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

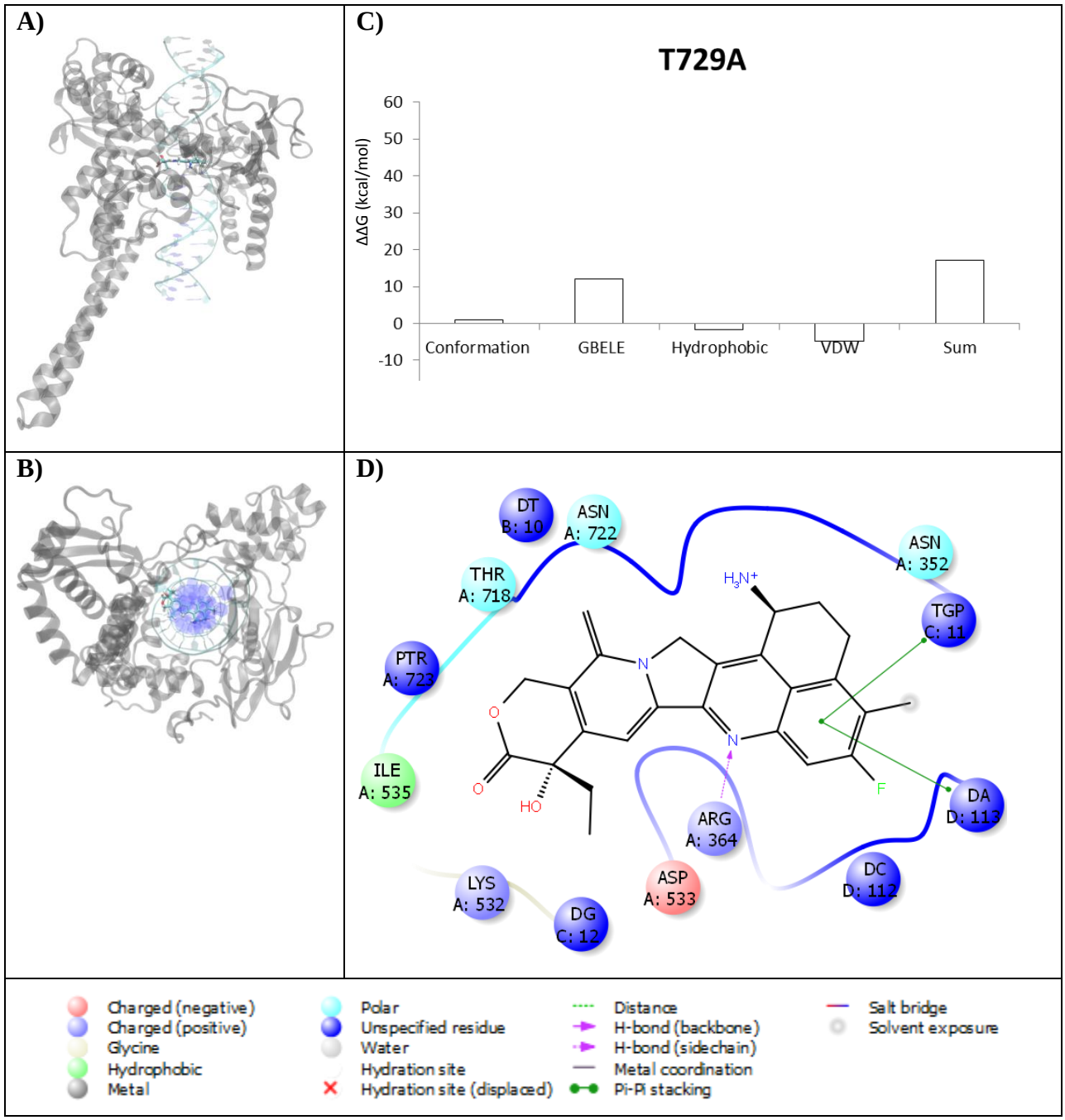


Figure **S54**. Exatecan Bound to Complex T279A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

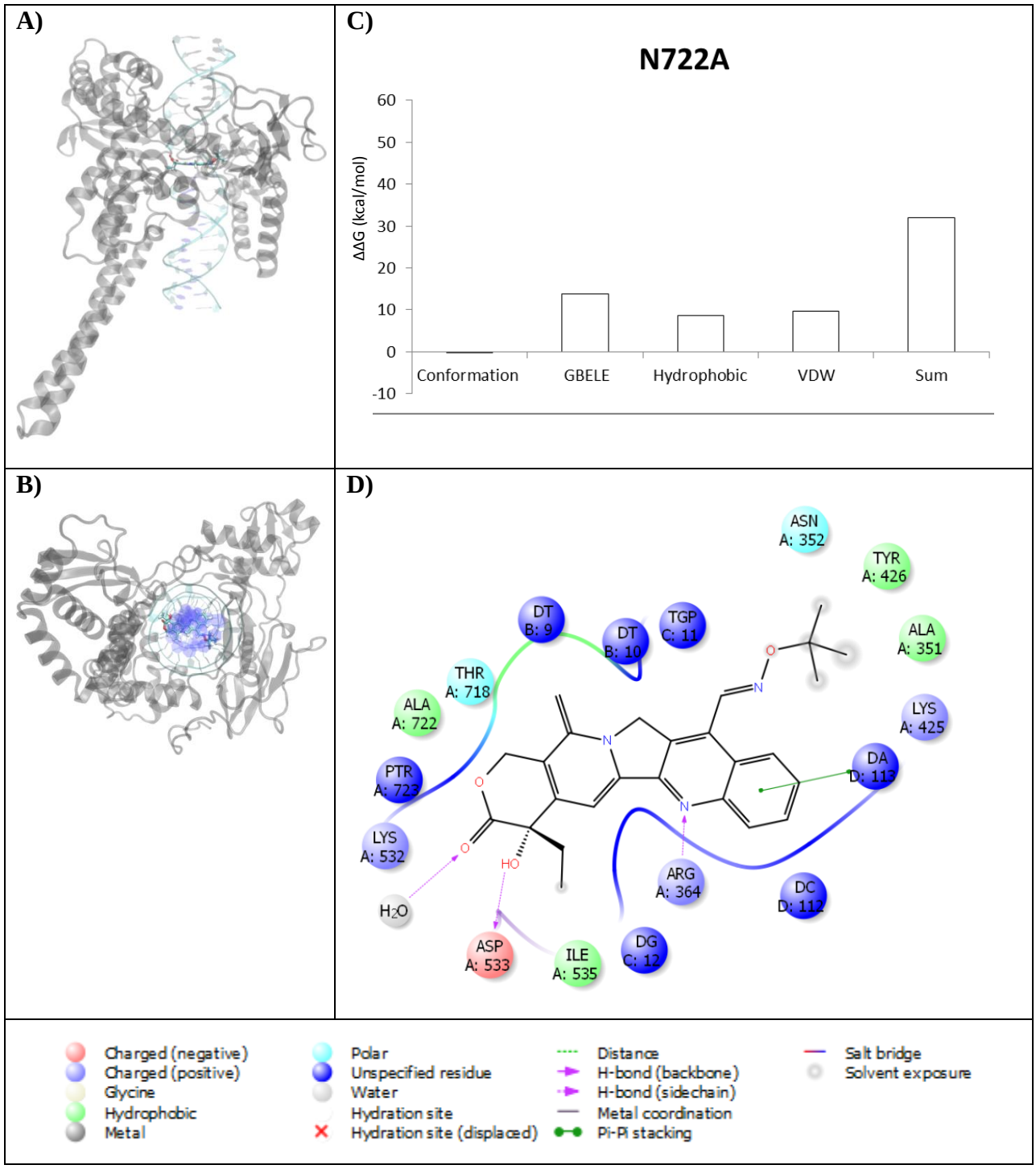


Figure **S55**. Gimatecan Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

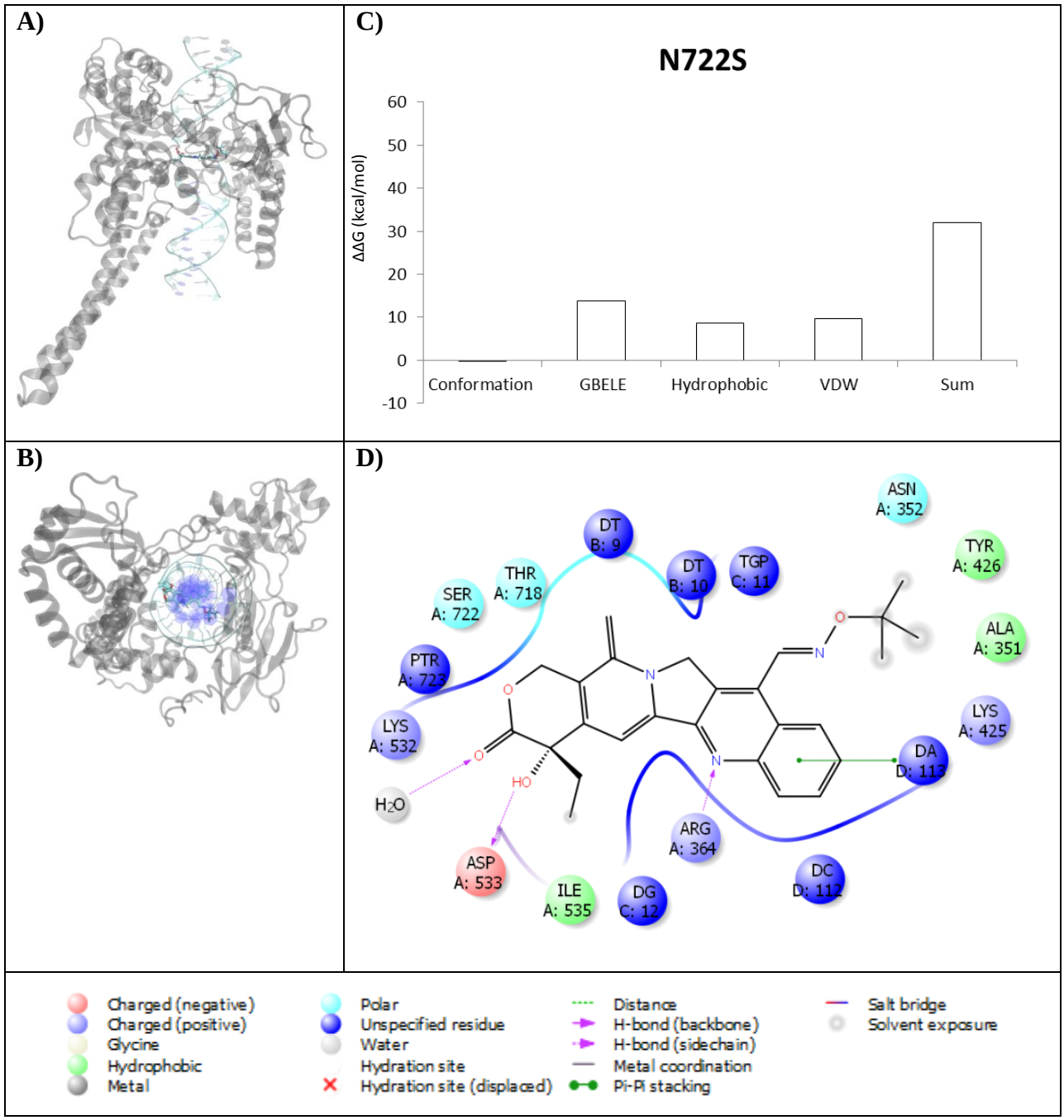


Figure **S56**. Gimatecan Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

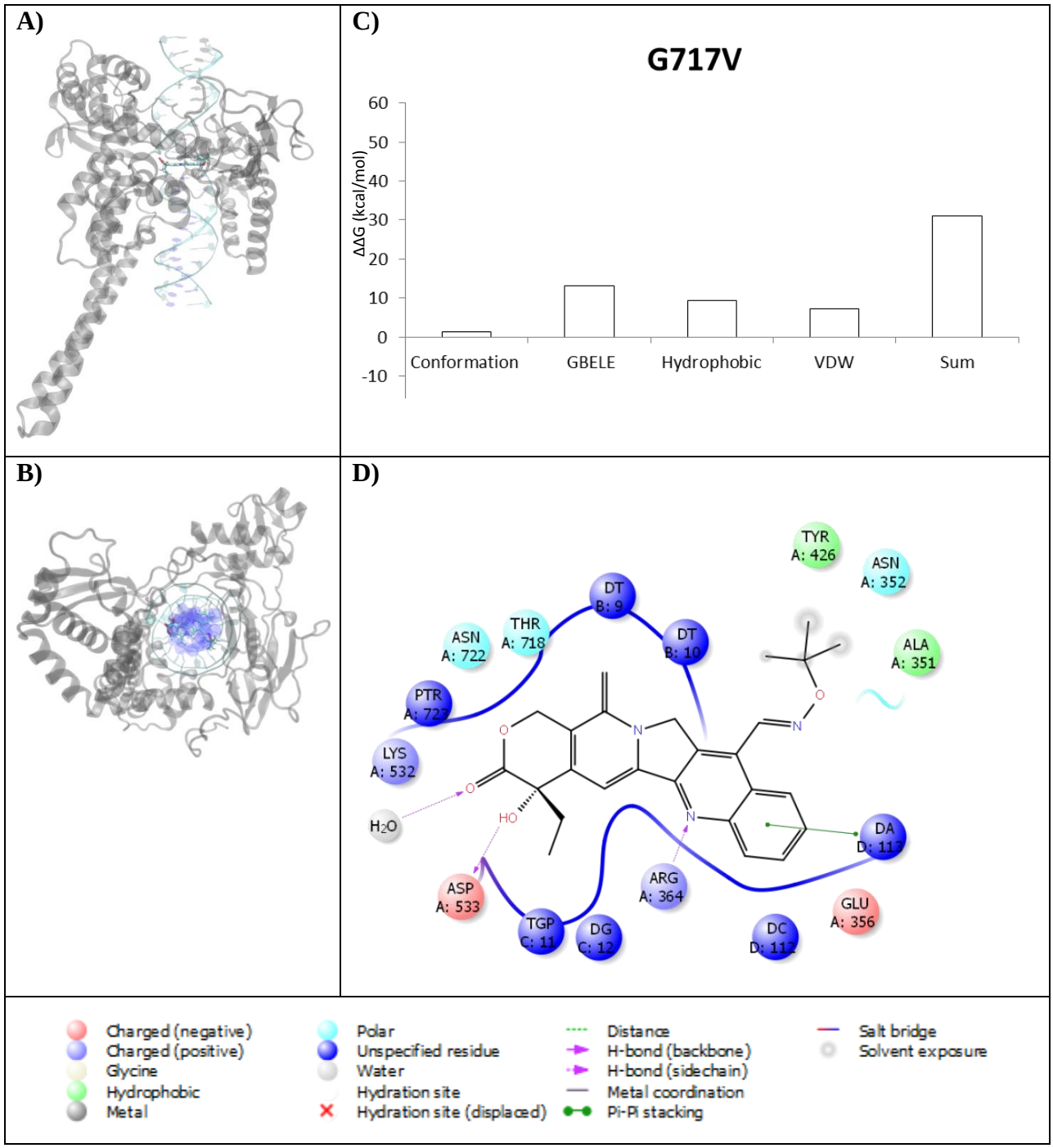


Figure **S57**. Gimatecan Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

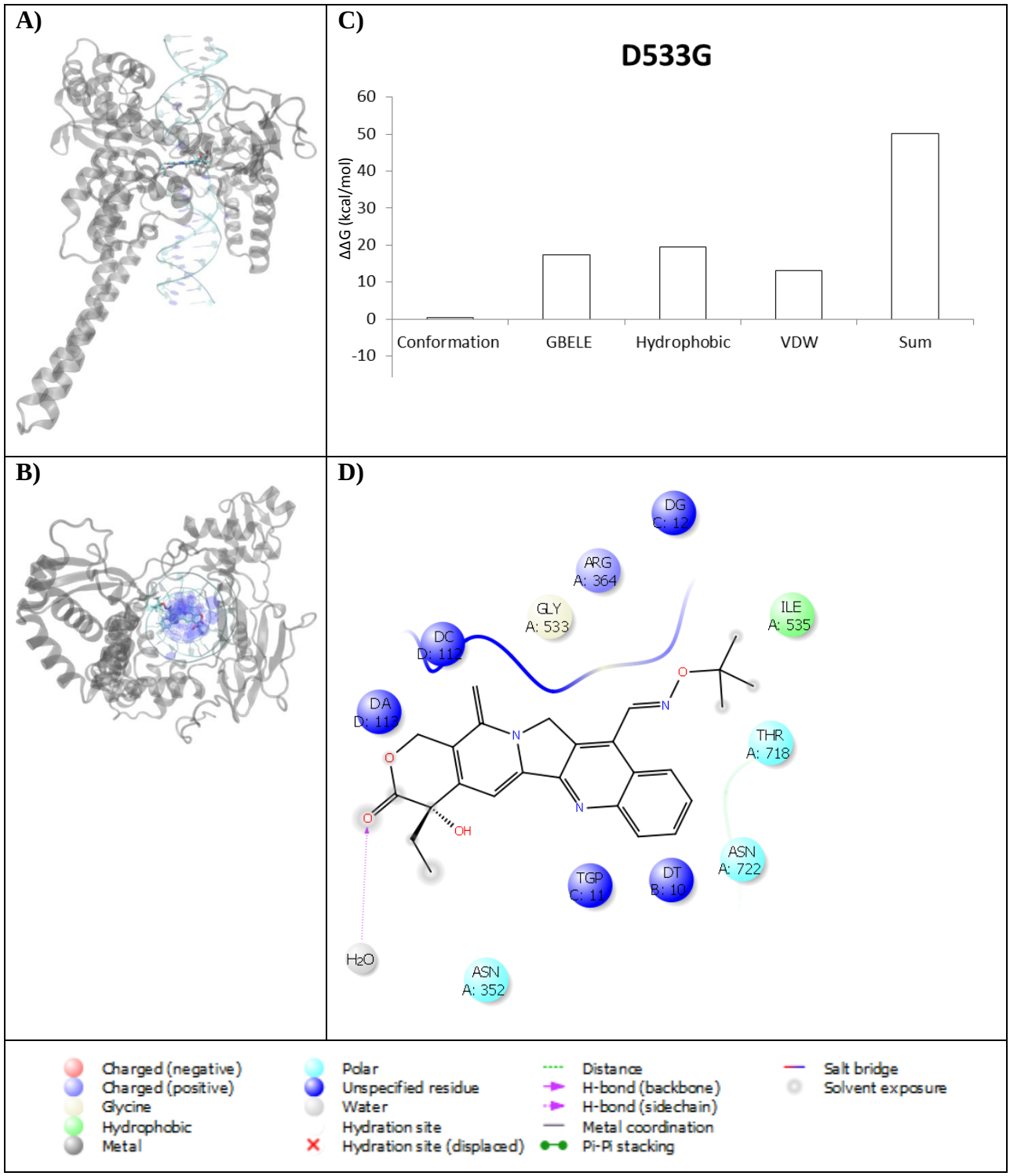


Figure **S58**. Gimatecan Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

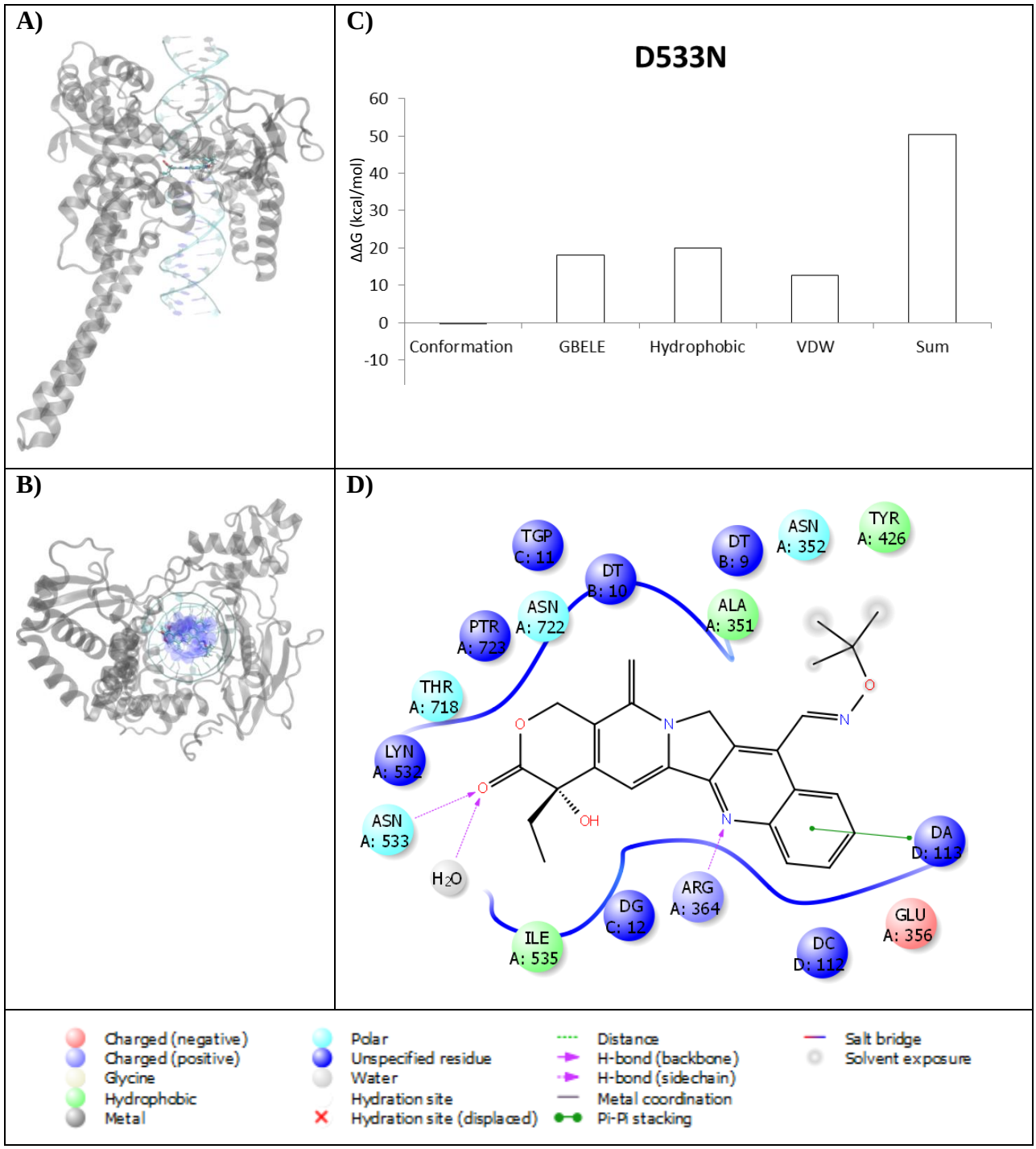


Figure **S59**. Gimatecan Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

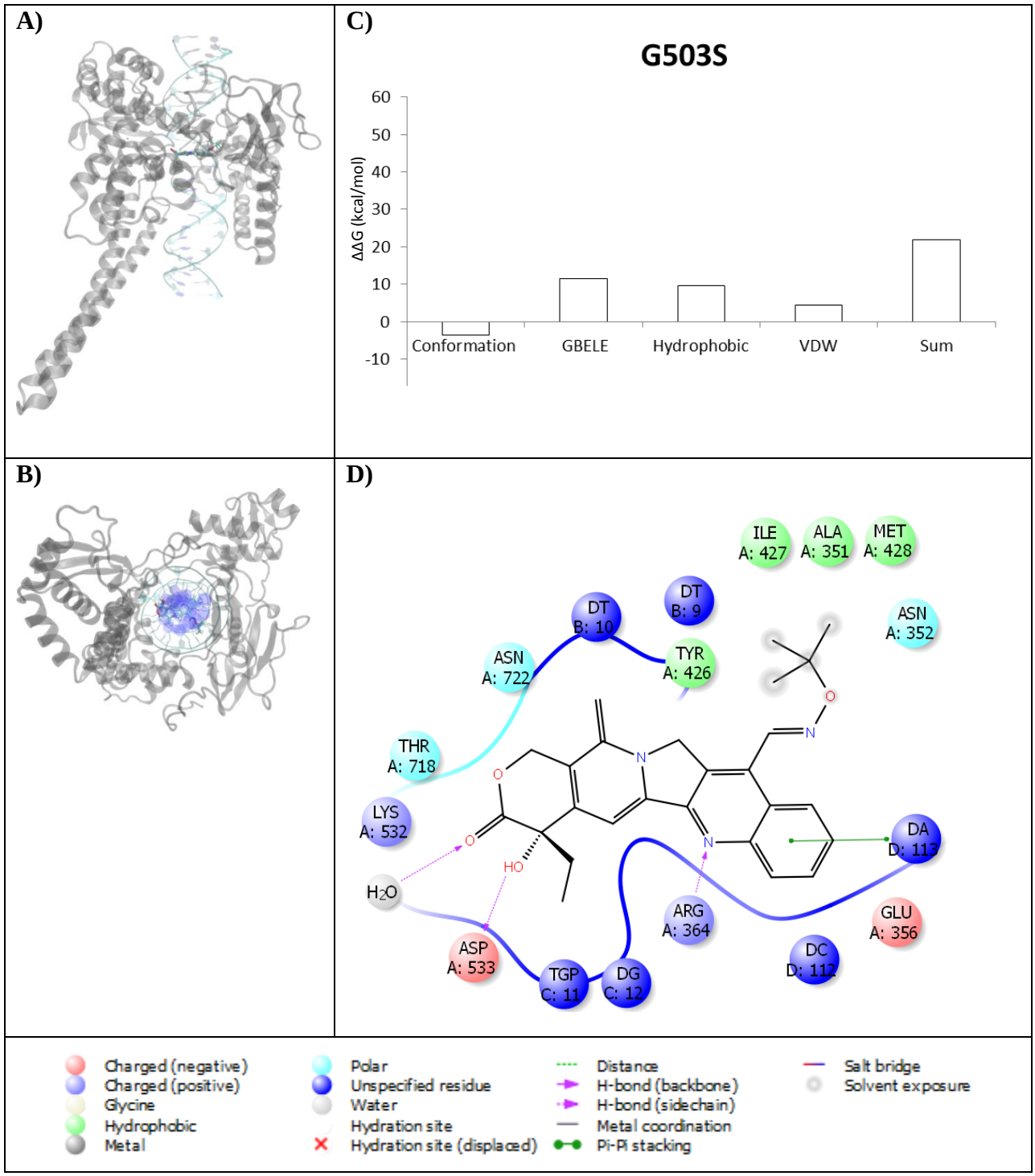


Figure **S60**. Gimatecan Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

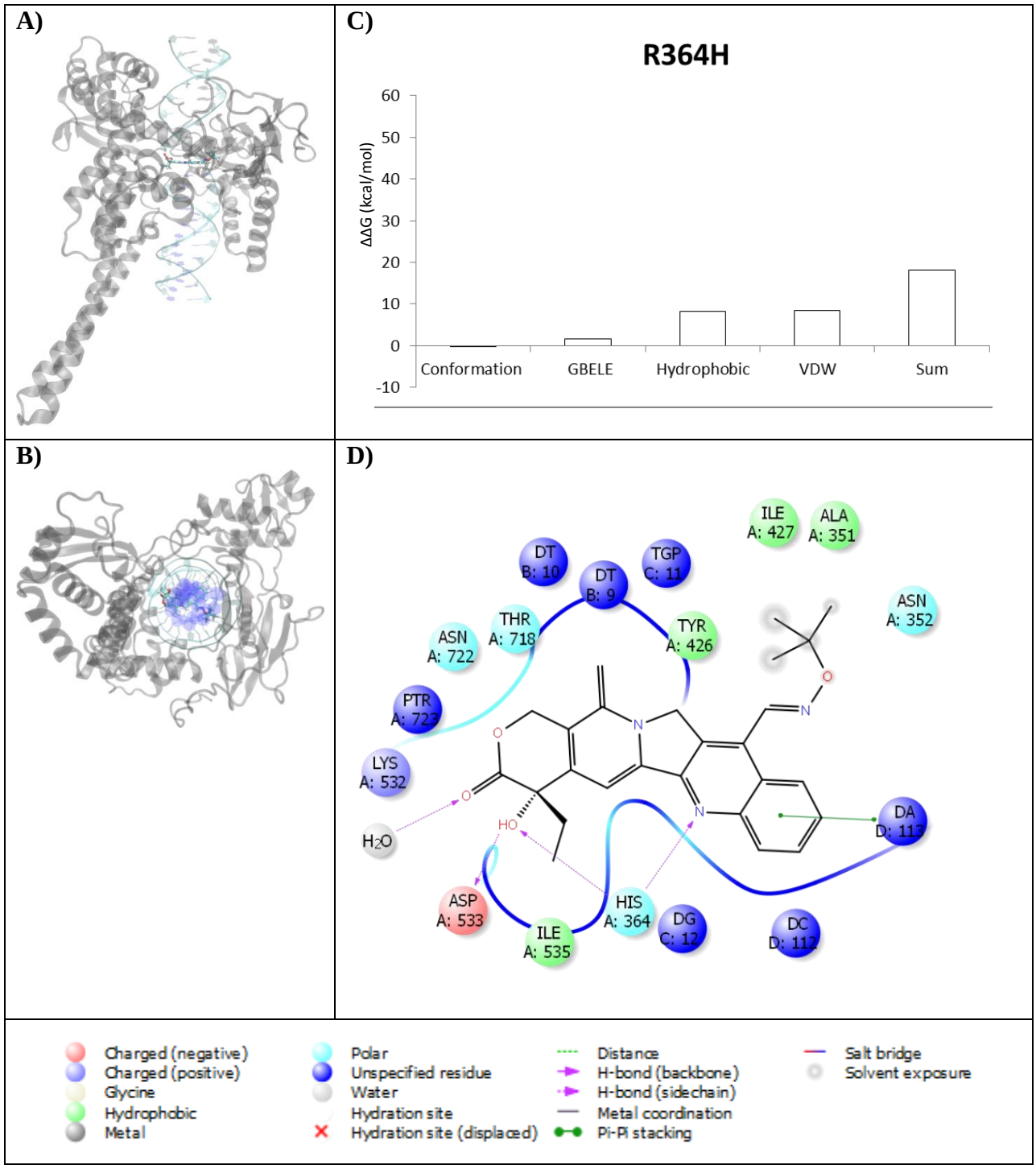


Figure **S61**. Gimatecan Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

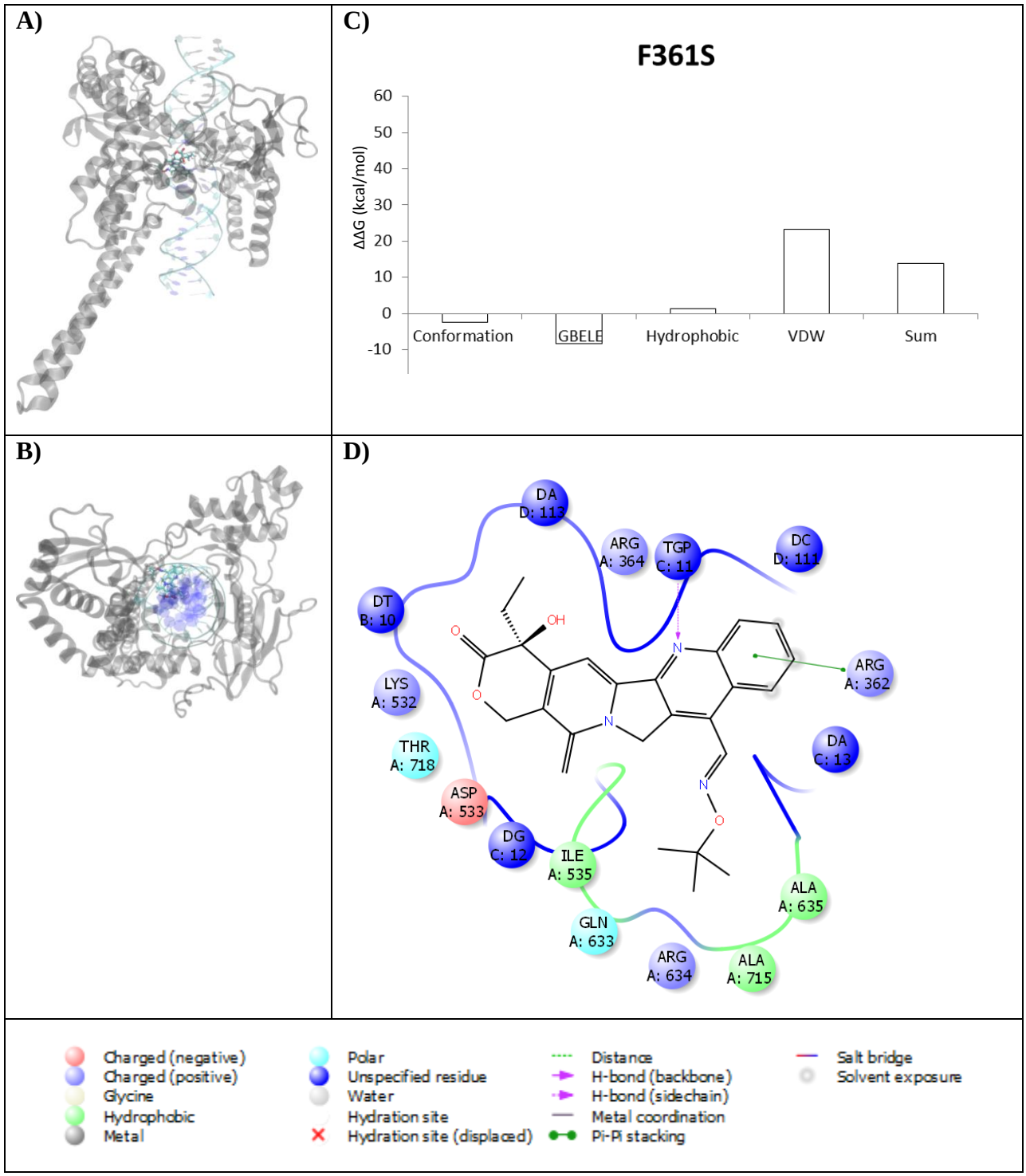


Figure **S62**. Gimatecan Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

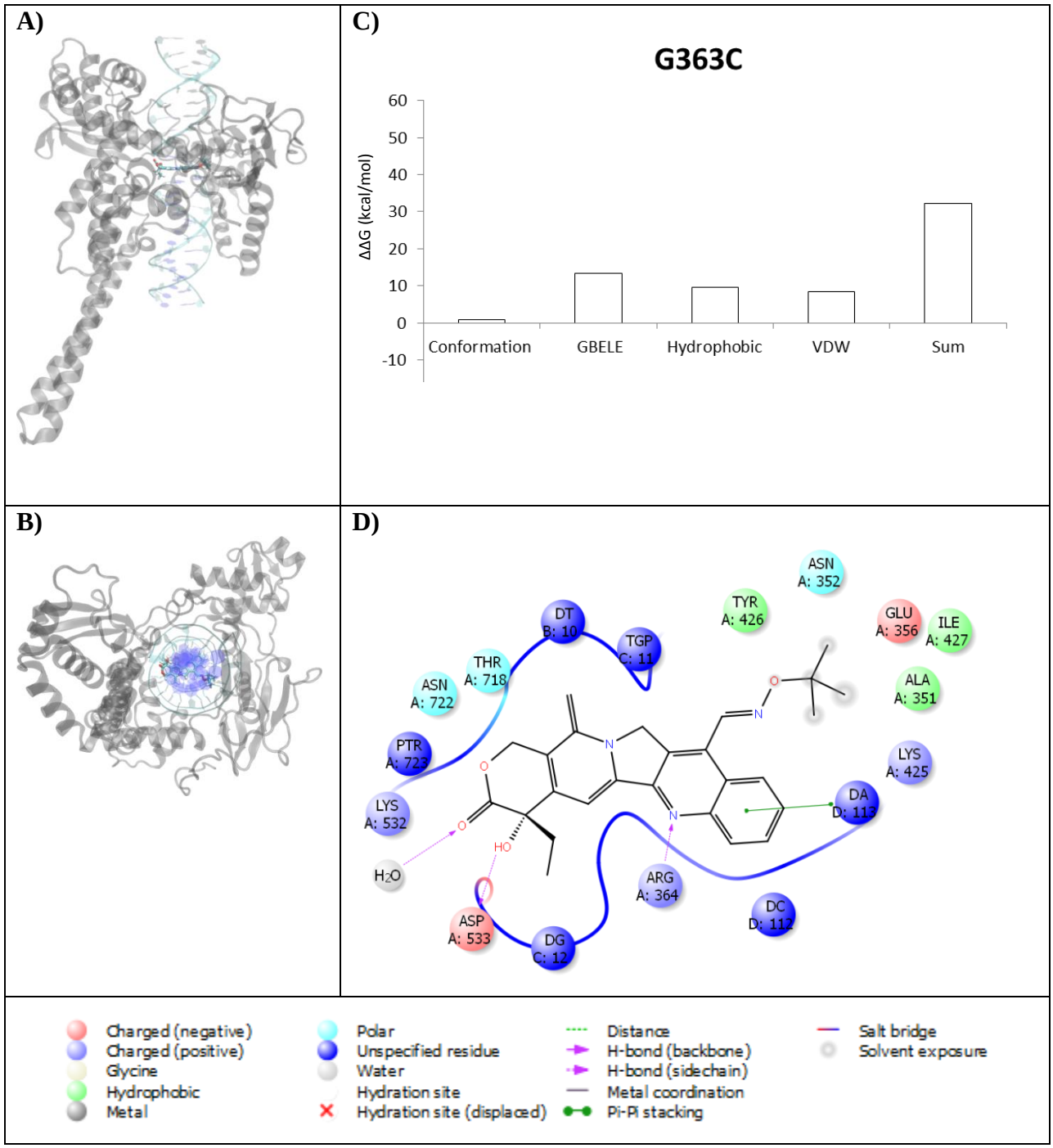


Figure **S63**. Gimatecan Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

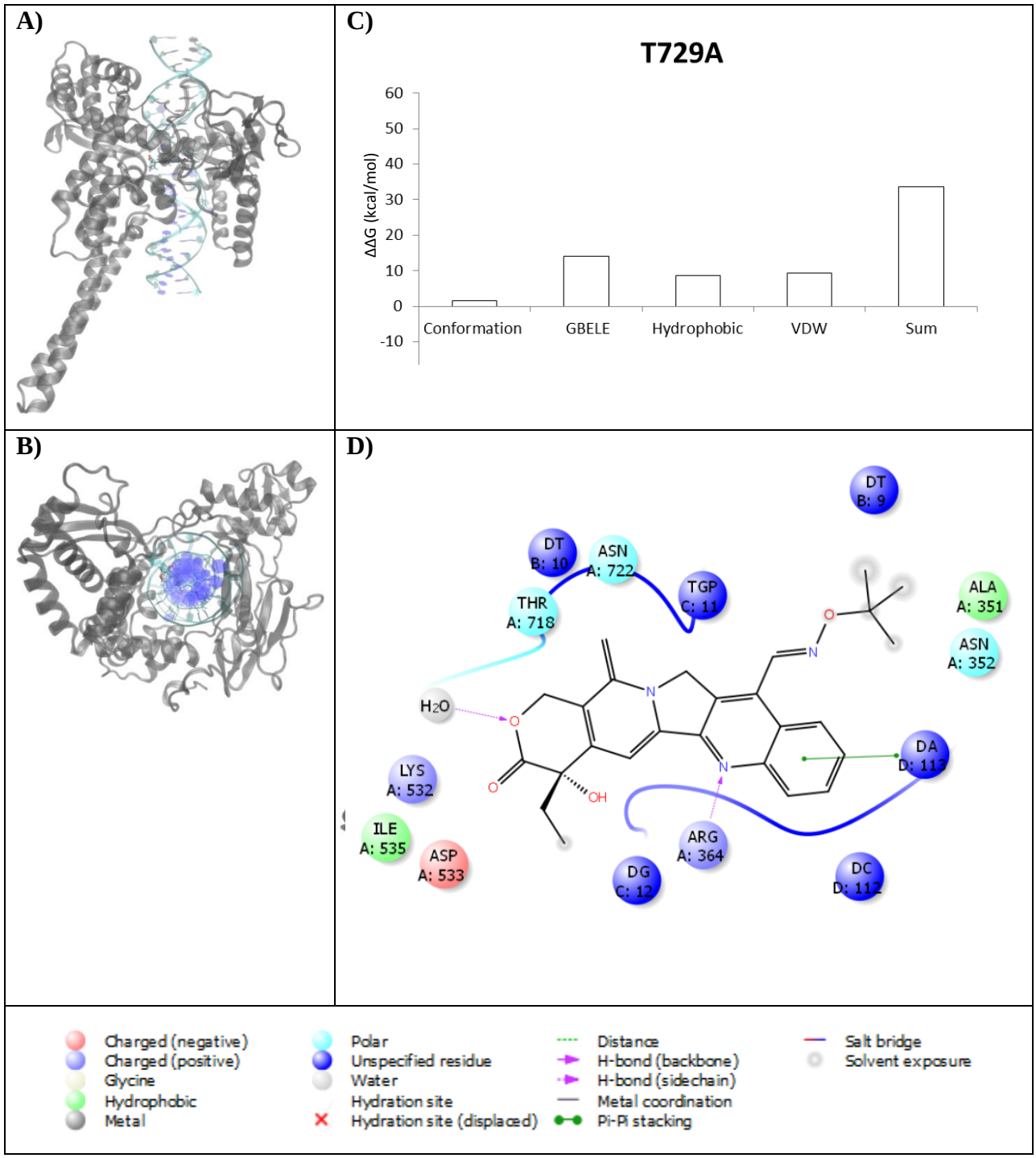


Figure **S64**. Gimatecan Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

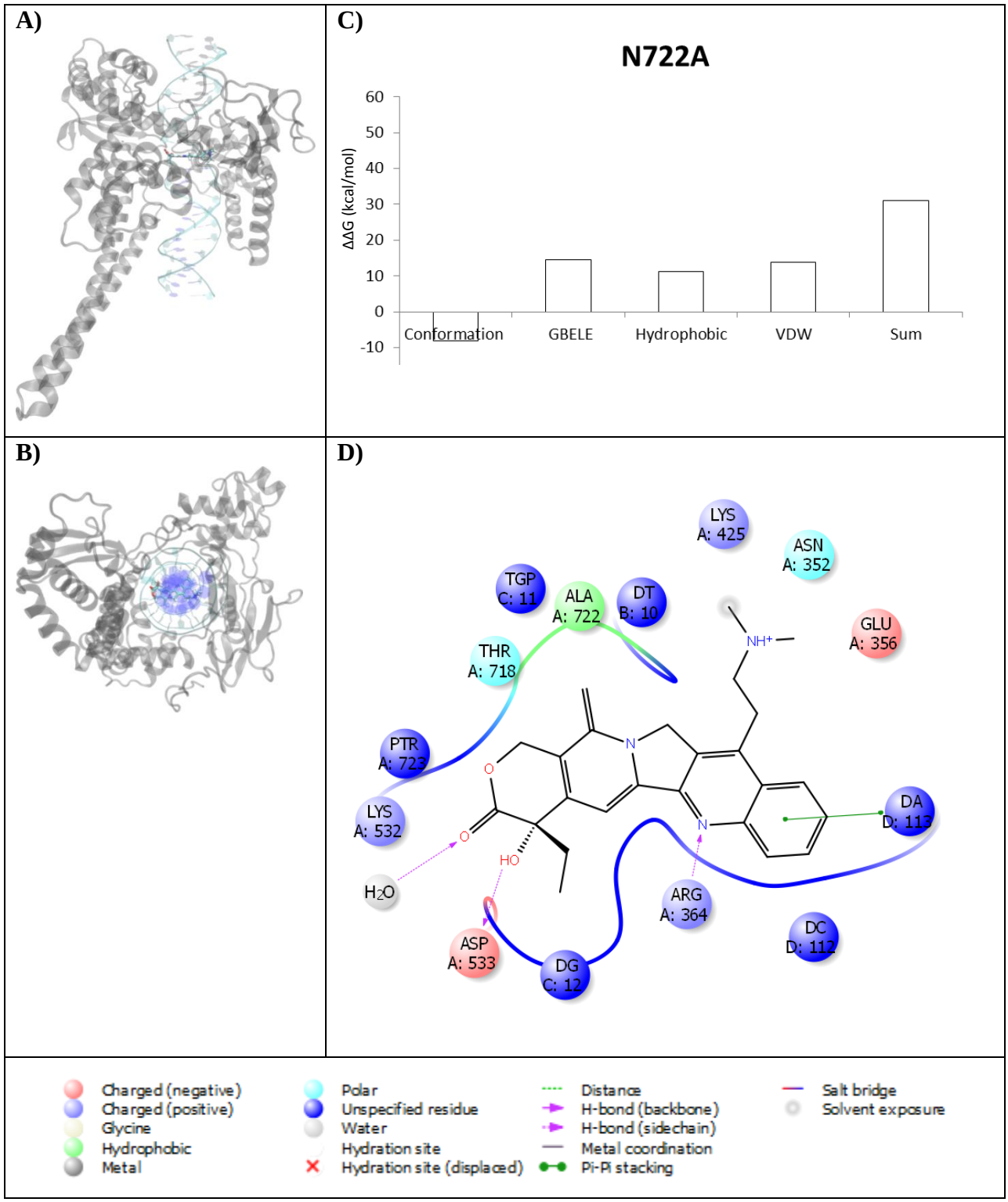


Figure **S65**. Belotecan Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

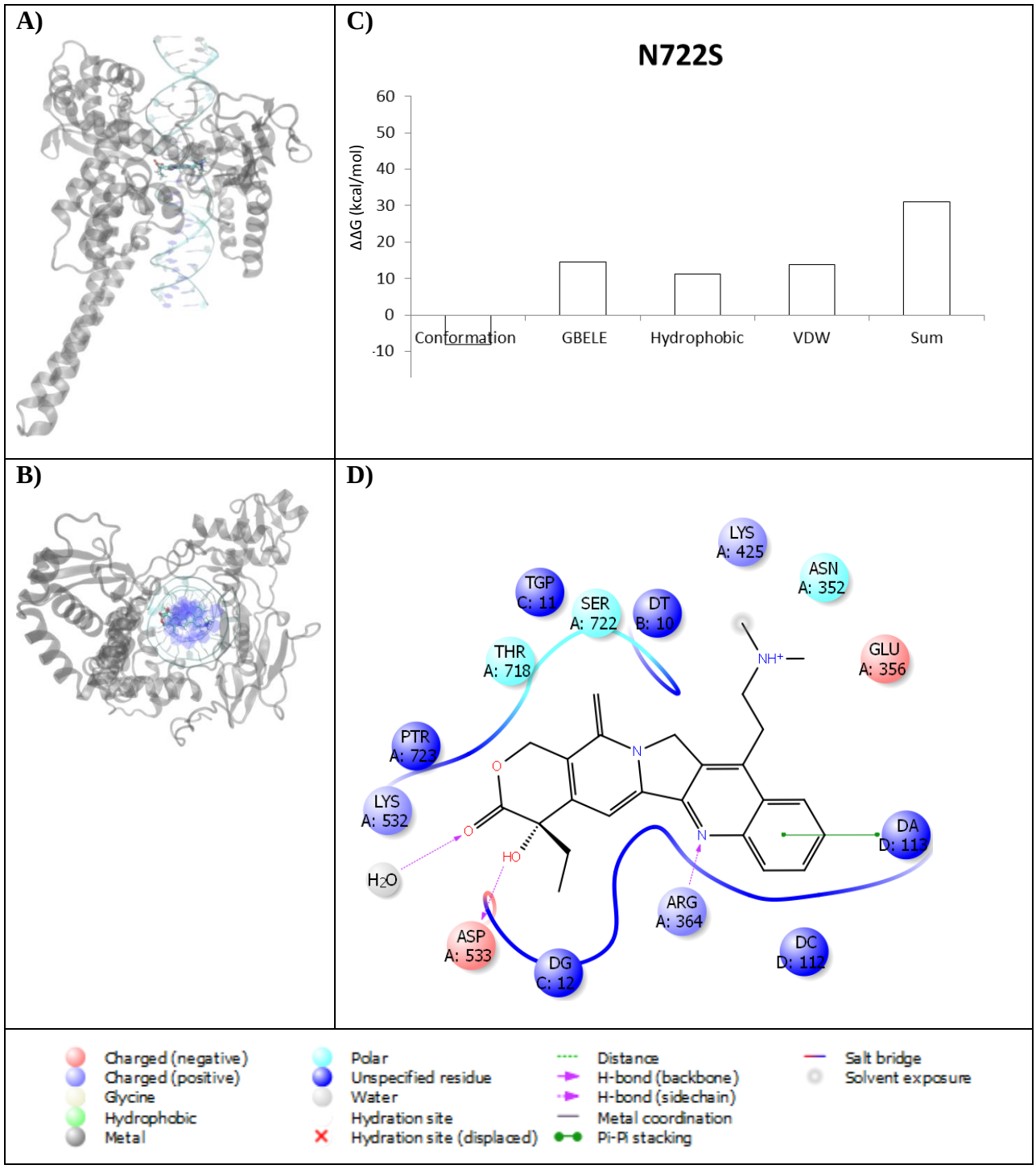


Figure **S66**. Belotecan Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

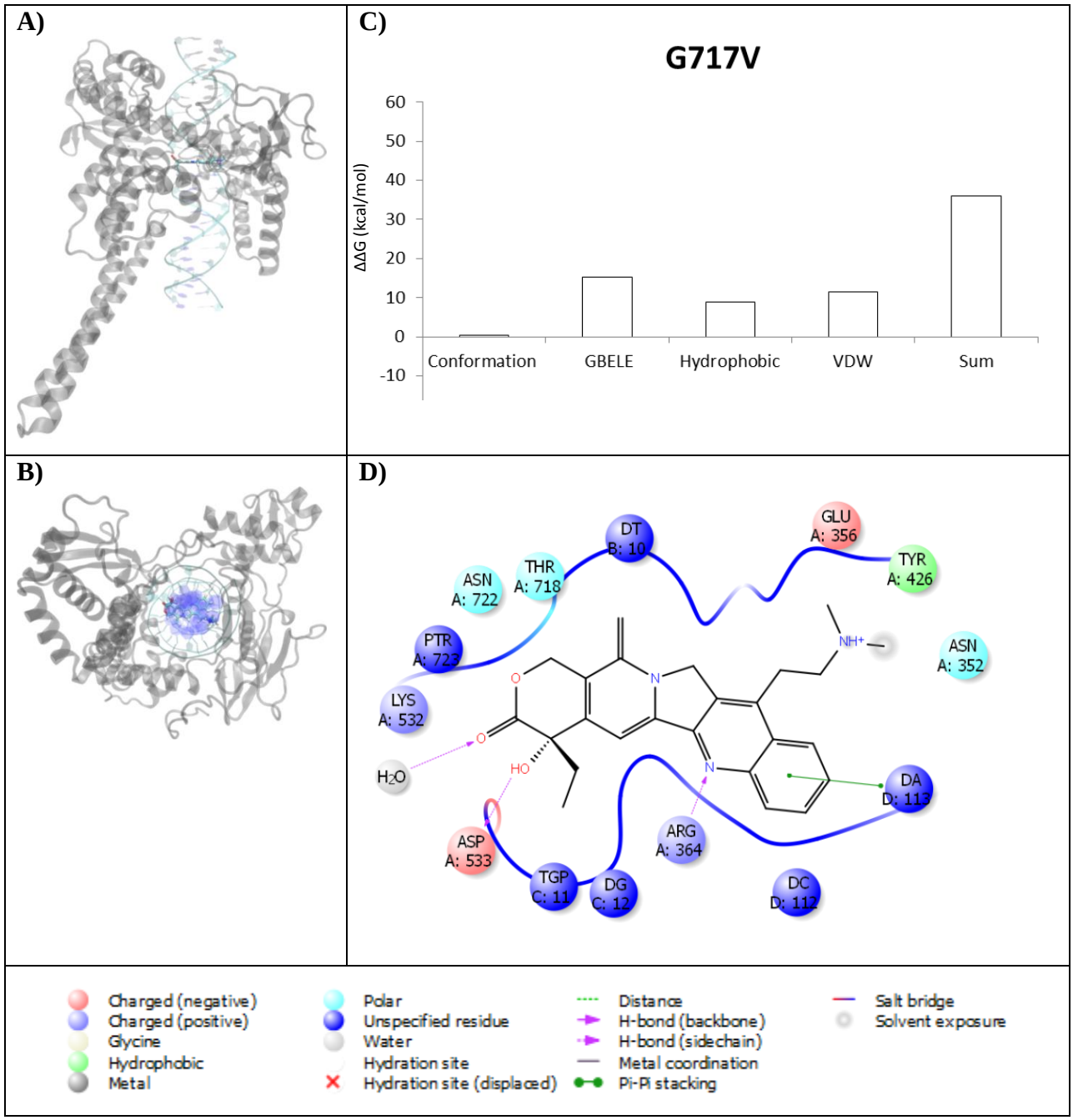


Figure **S67**. Belotecan Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

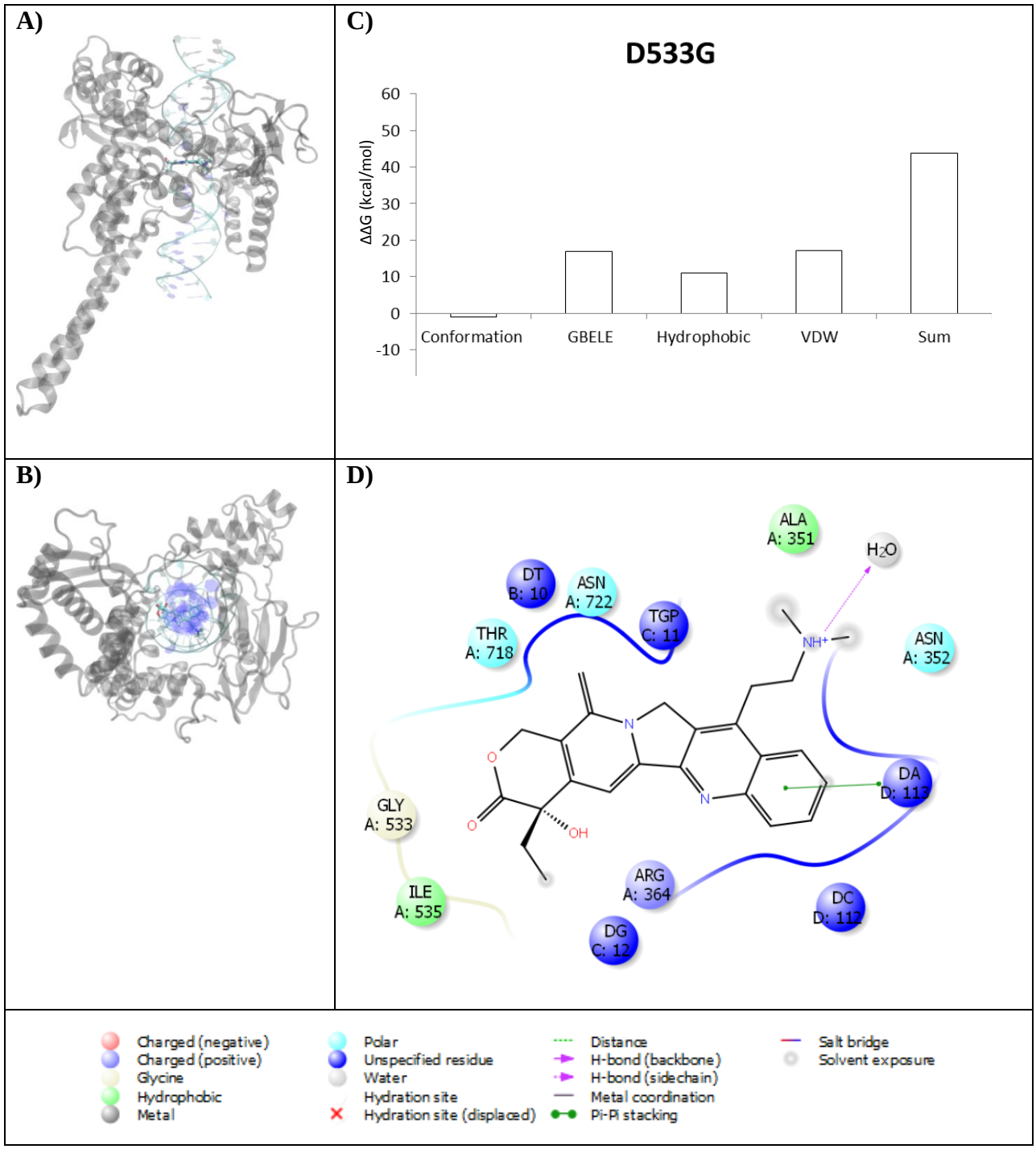


Figure **S68**. Belotecan Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

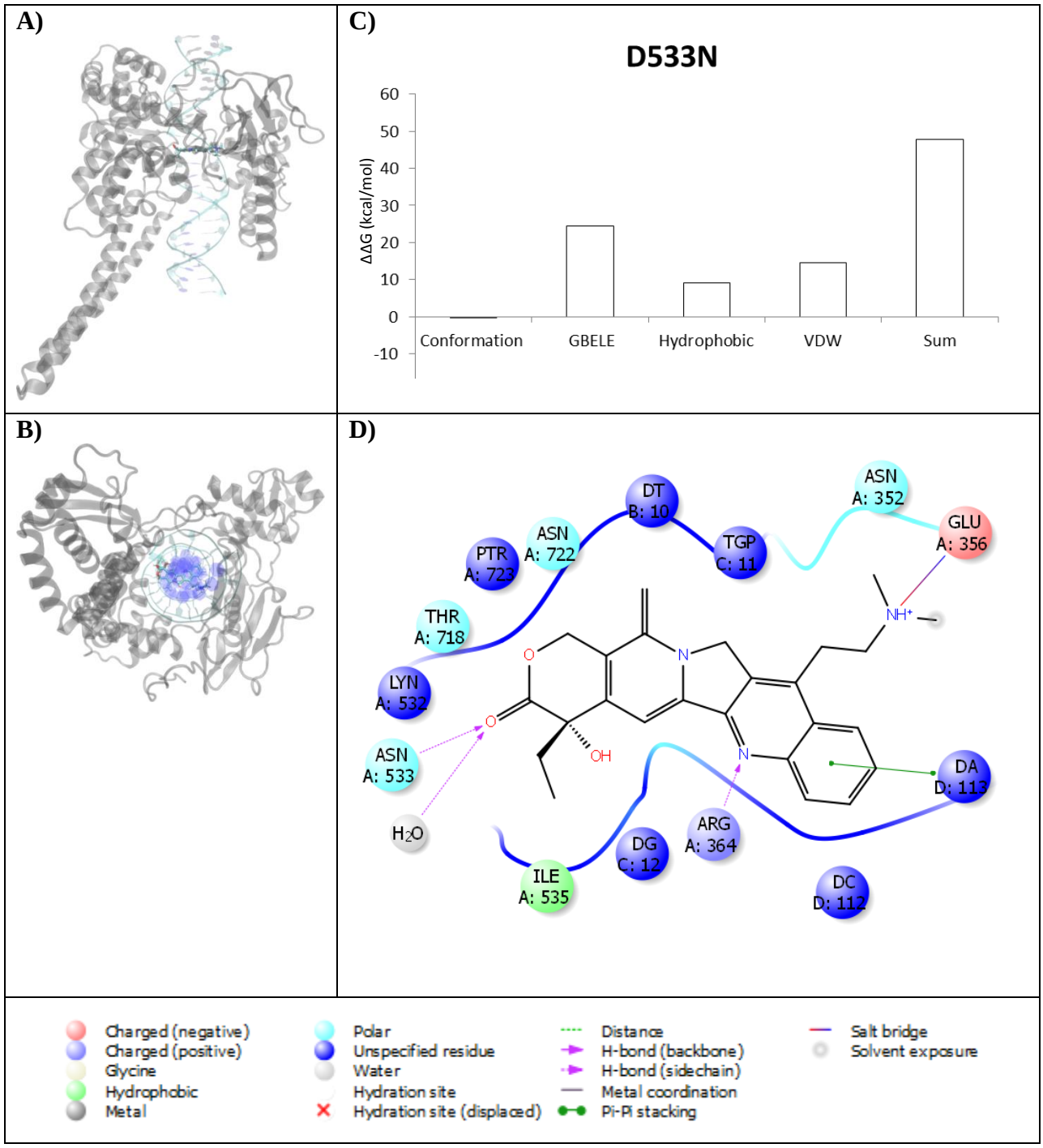


Figure **S69**. Belotecan Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

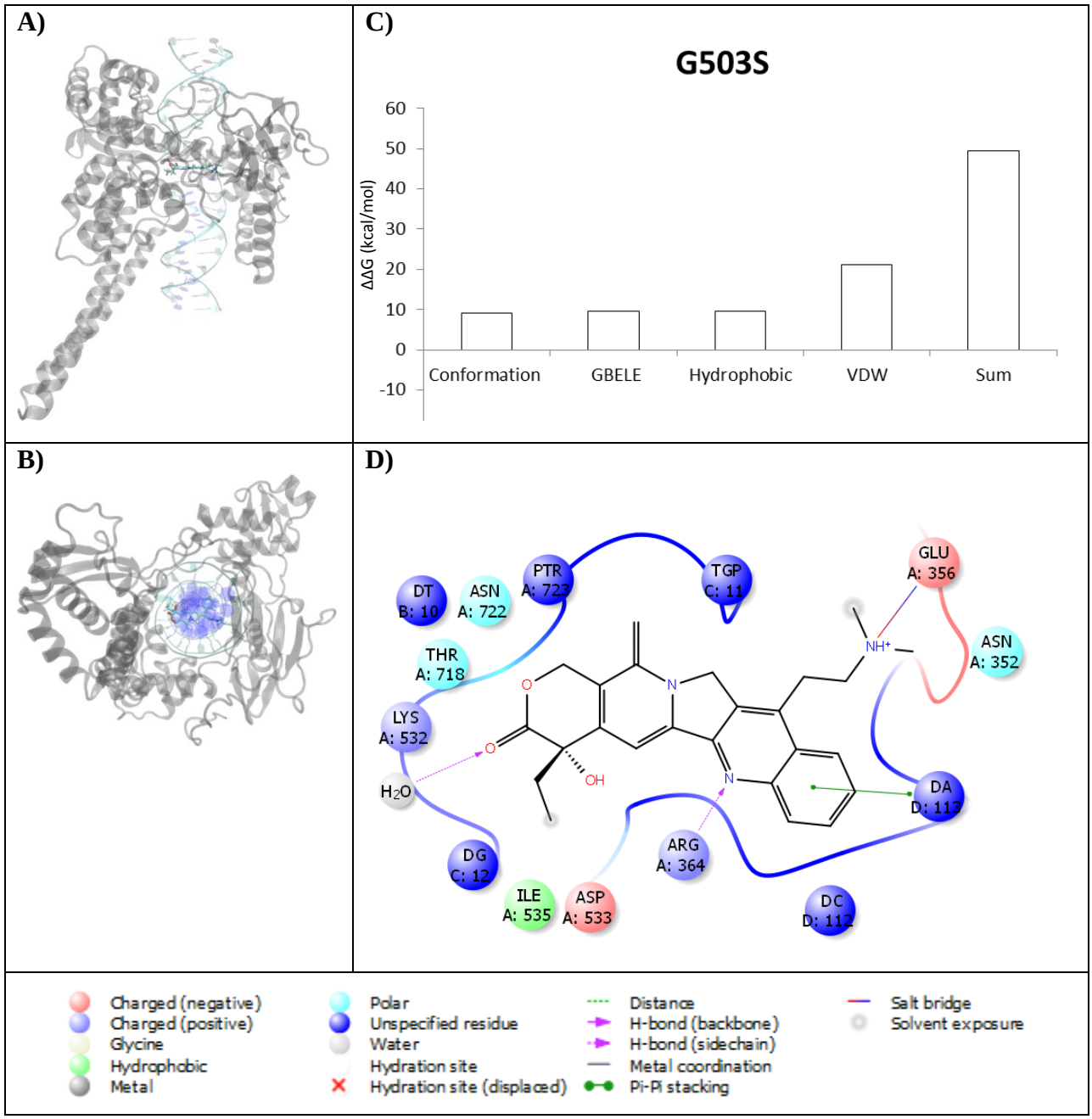


Figure **S70**. Belotecan Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

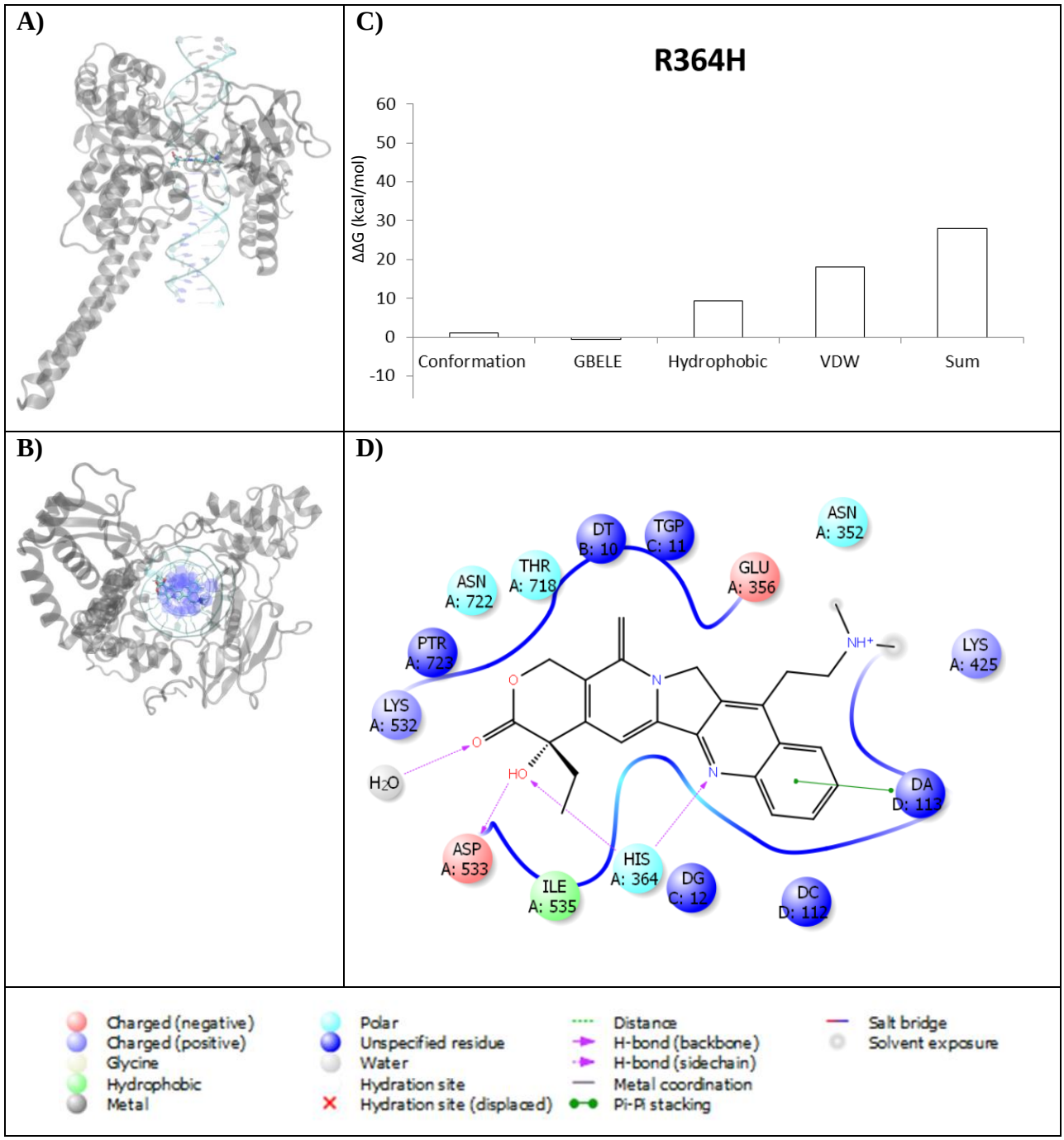


Figure **S71**. Belotecan Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

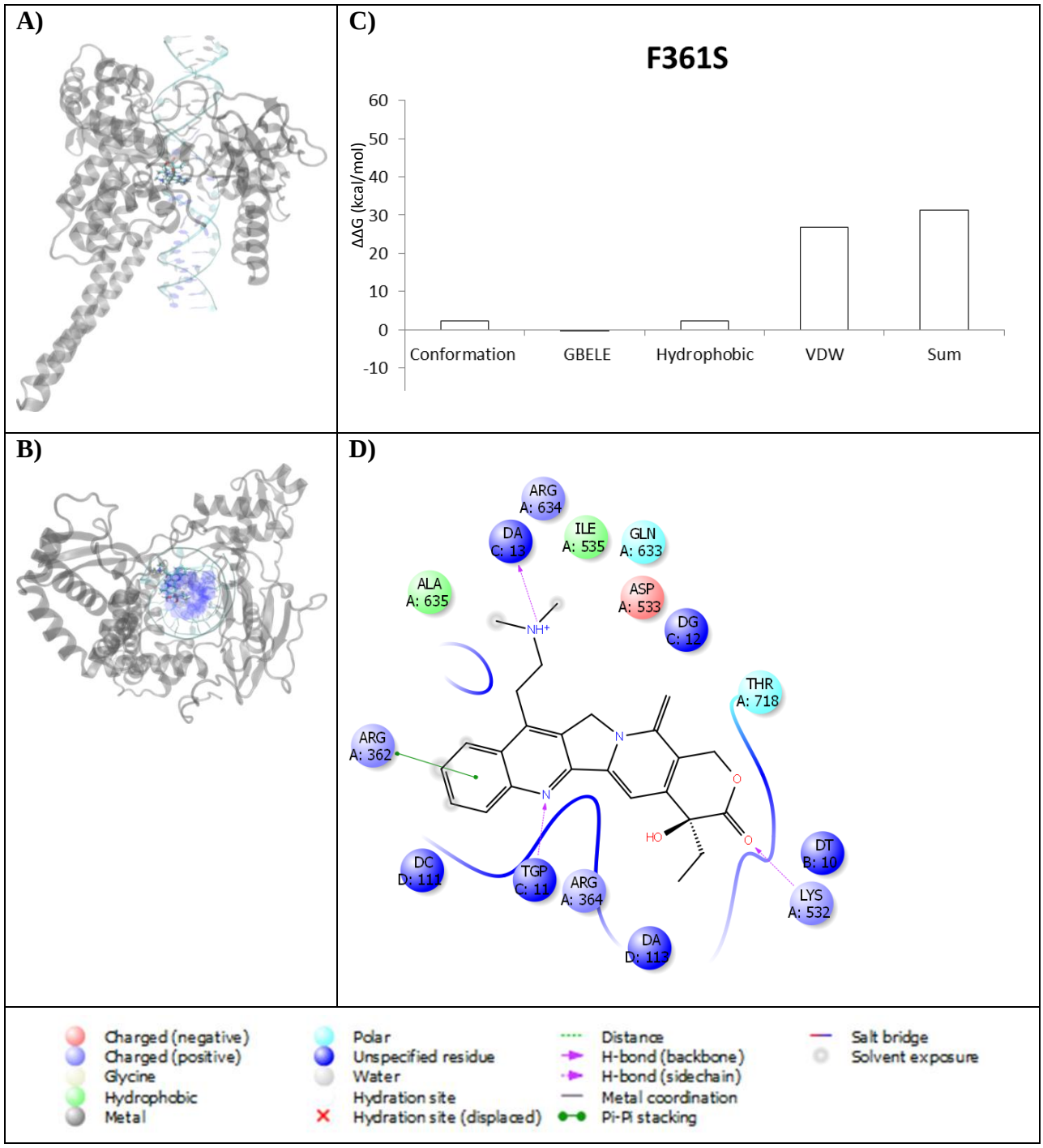


Figure **S72**. Belotecan Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

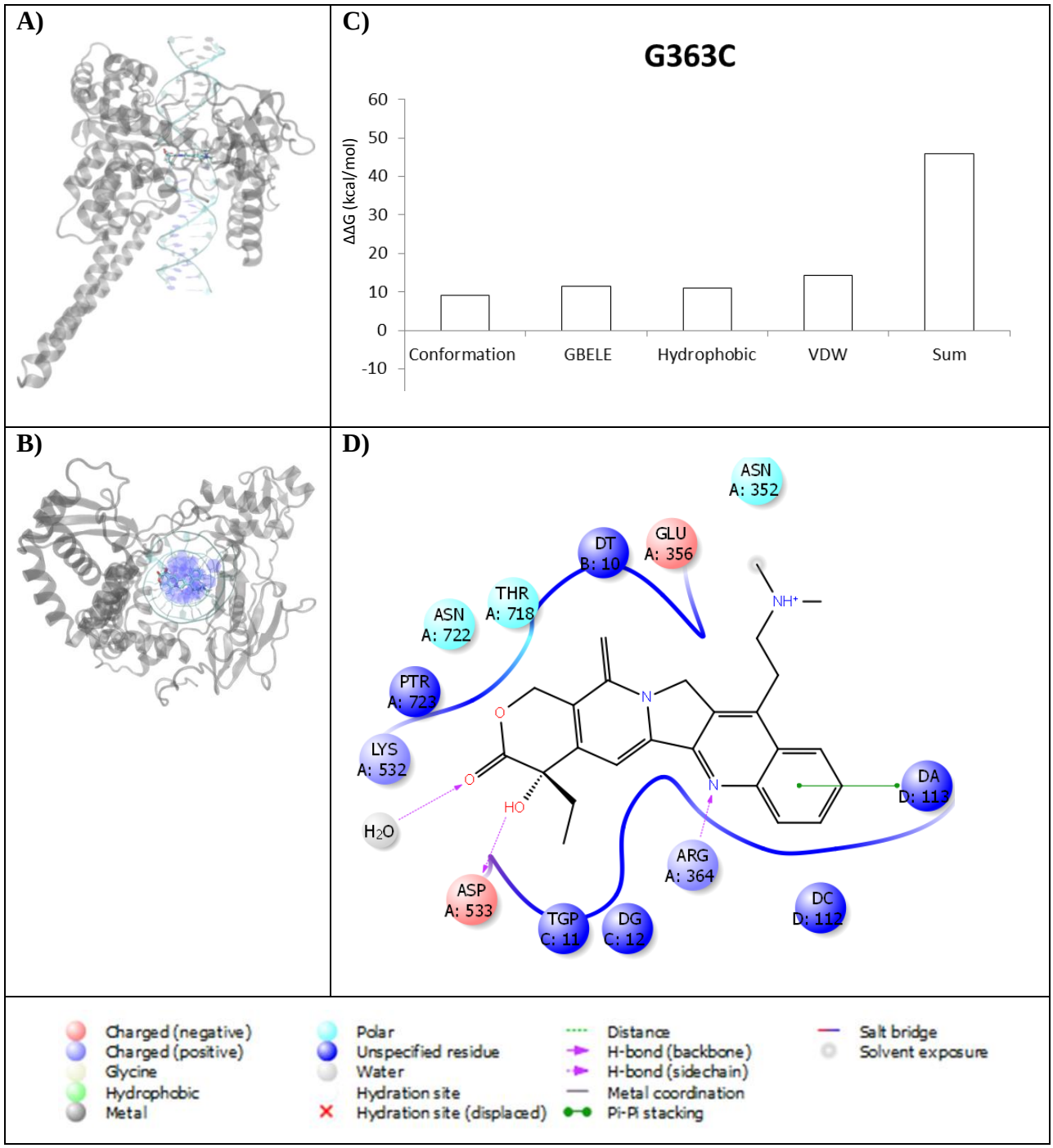


Figure **S73**. Belotecan Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

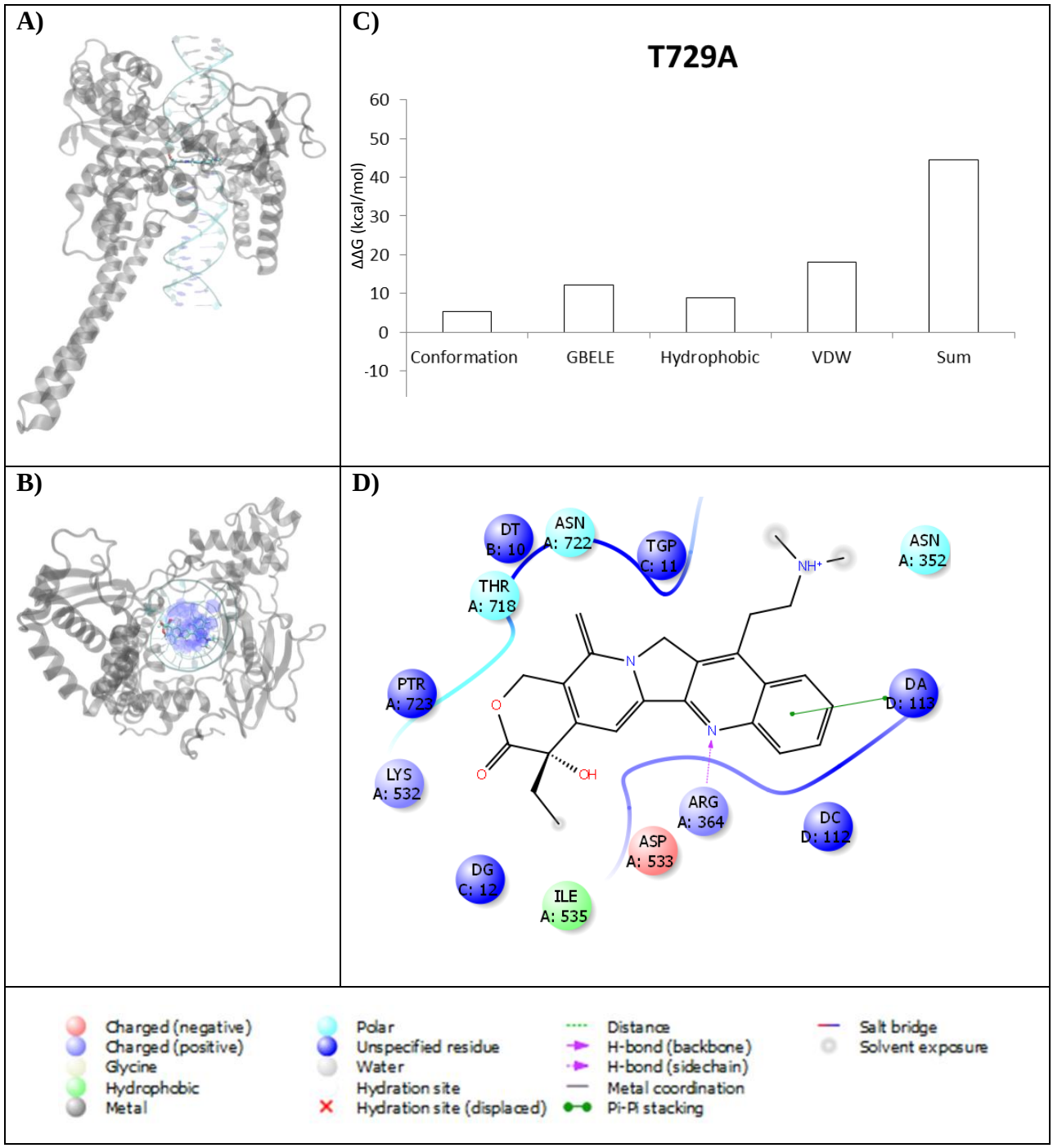


Figure **S74**. Belotecan Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

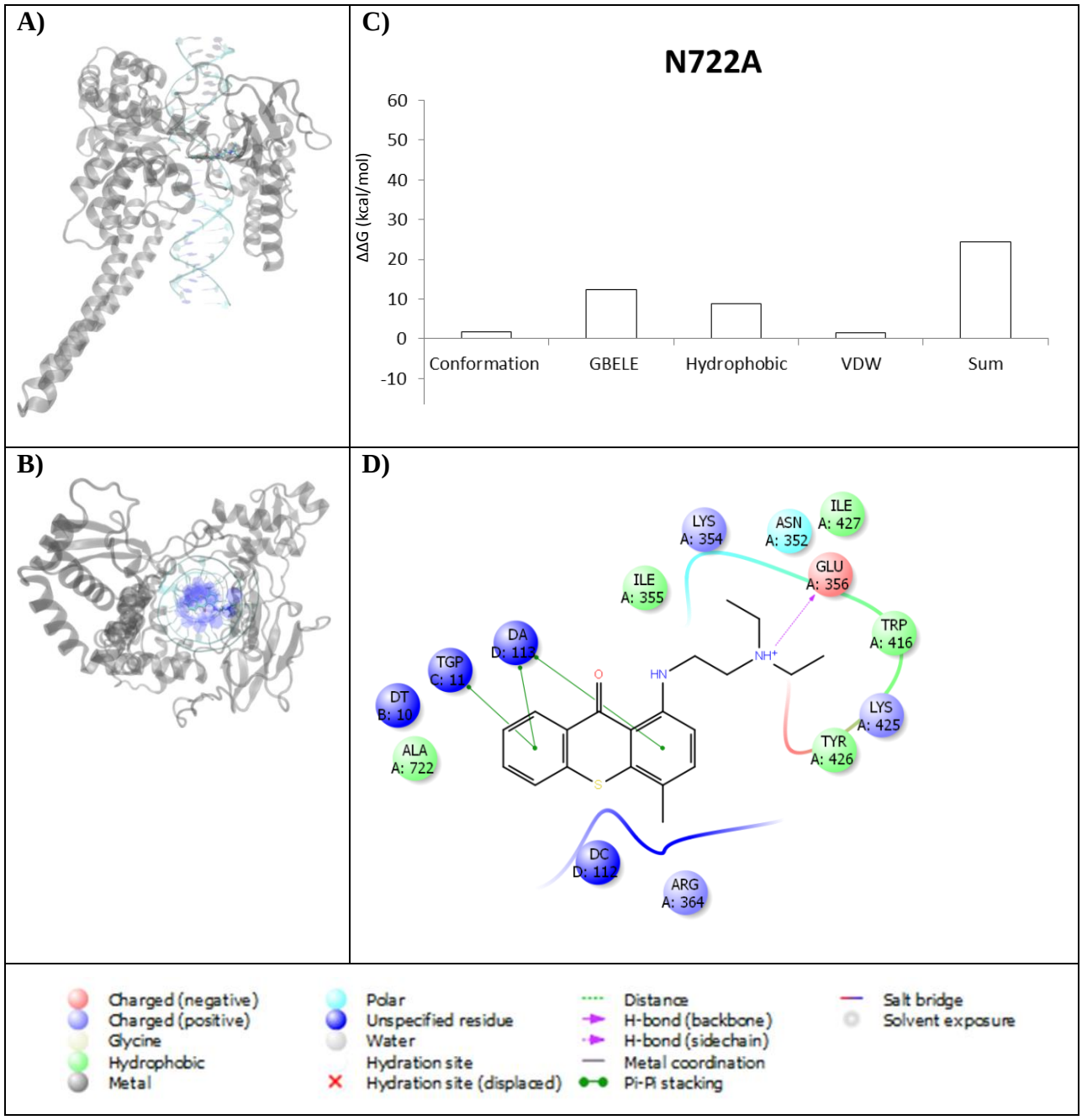


Figure **S75**. Lucanthone Bound to Complex N722A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

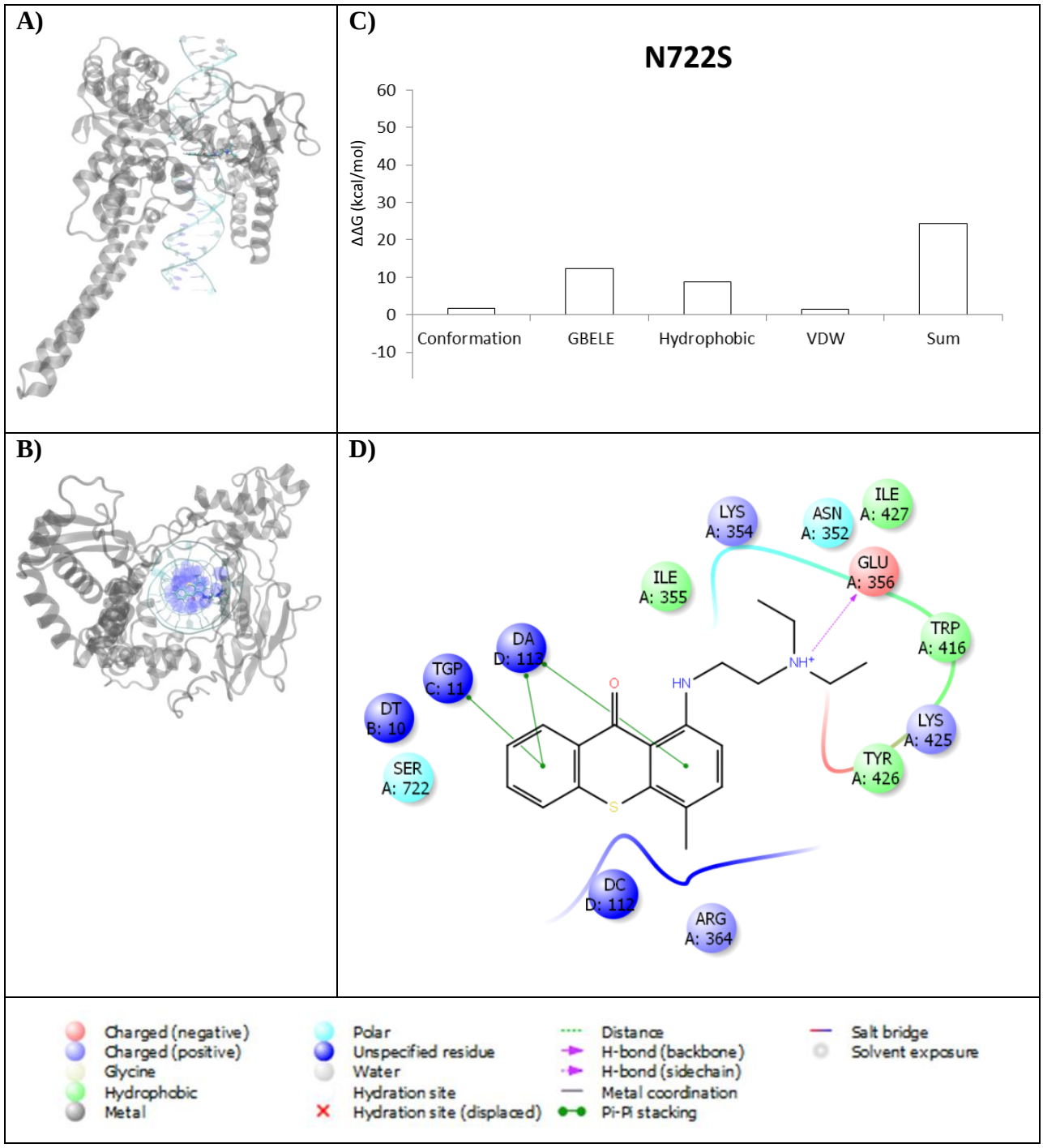


Figure **S76**. Lucanthone Bound to Complex N722S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

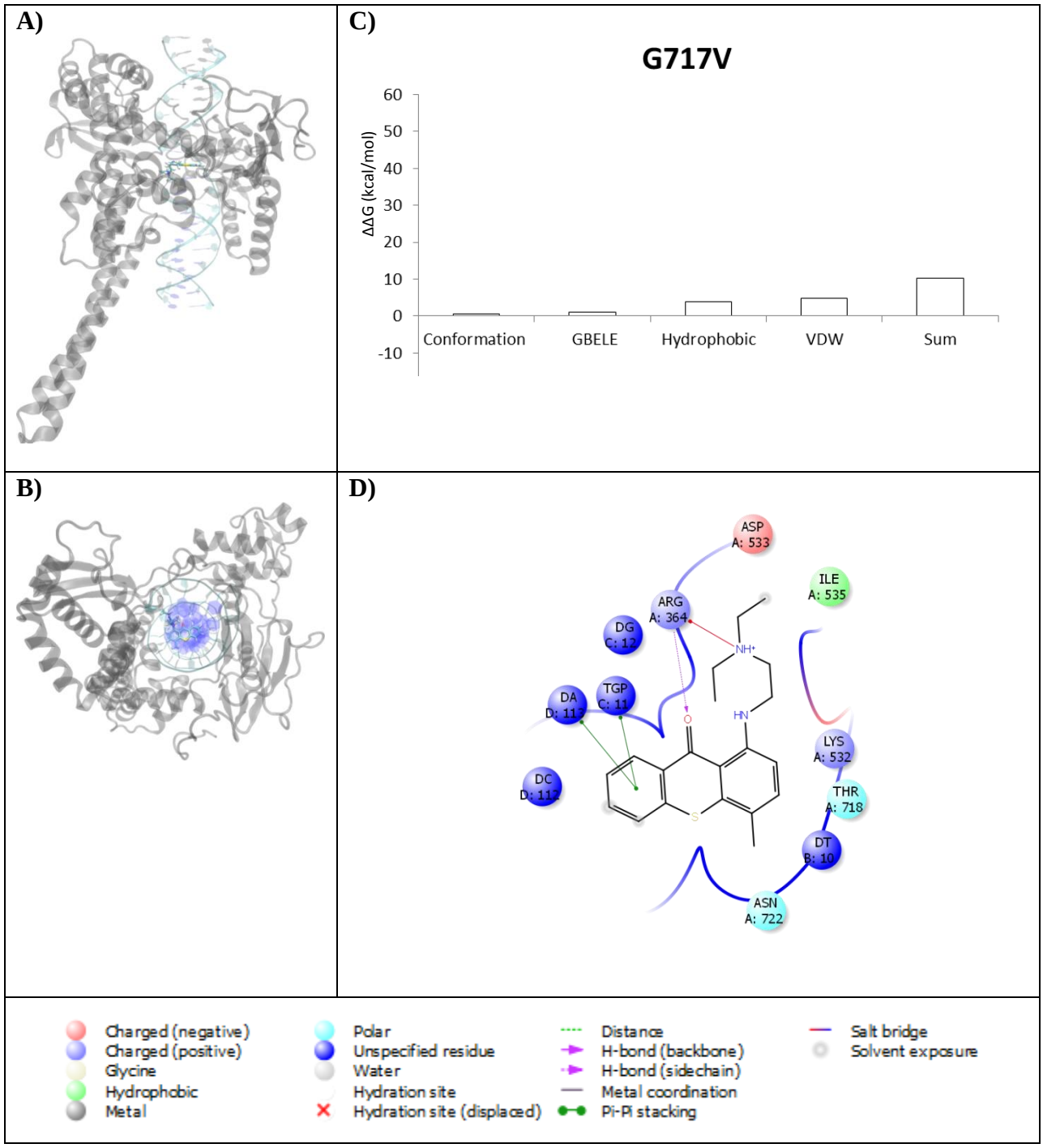


Figure **S77**. Lucanthone Bound to Complex G717V. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

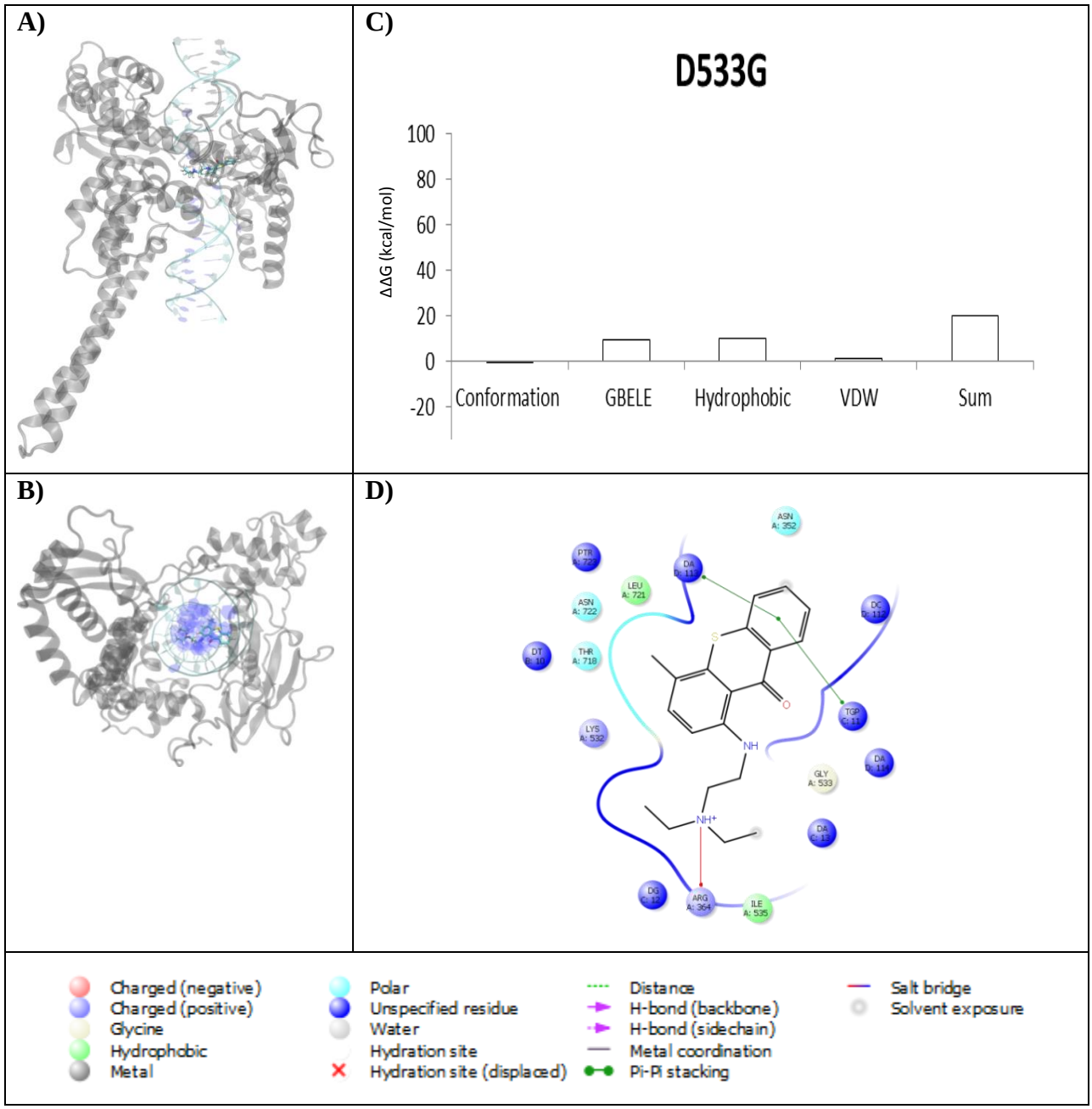


Figure **S78**. Lucanthone Bound to Complex D533G. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

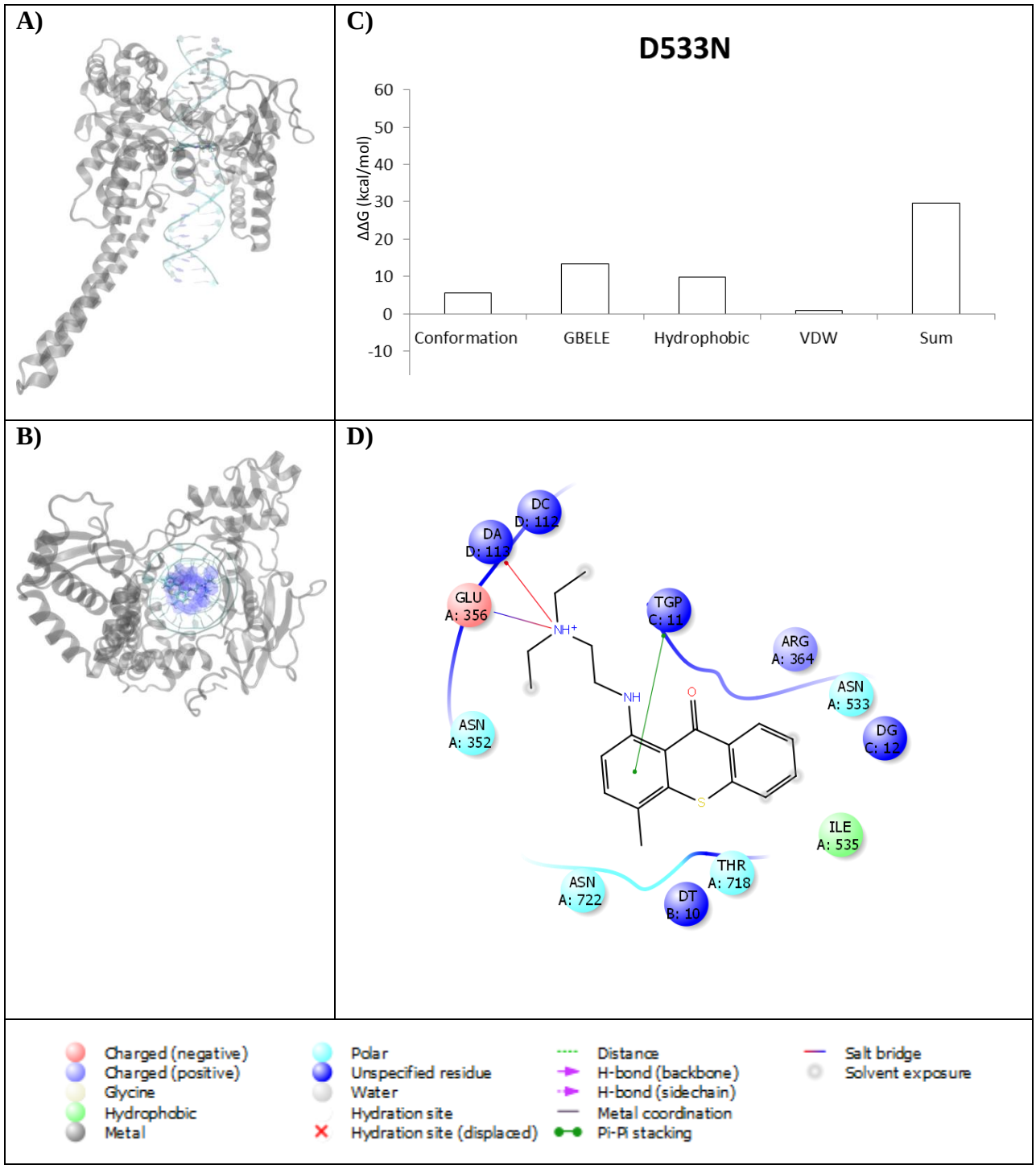


Figure **S79**. Lucanthone Bound to Complex D533N. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

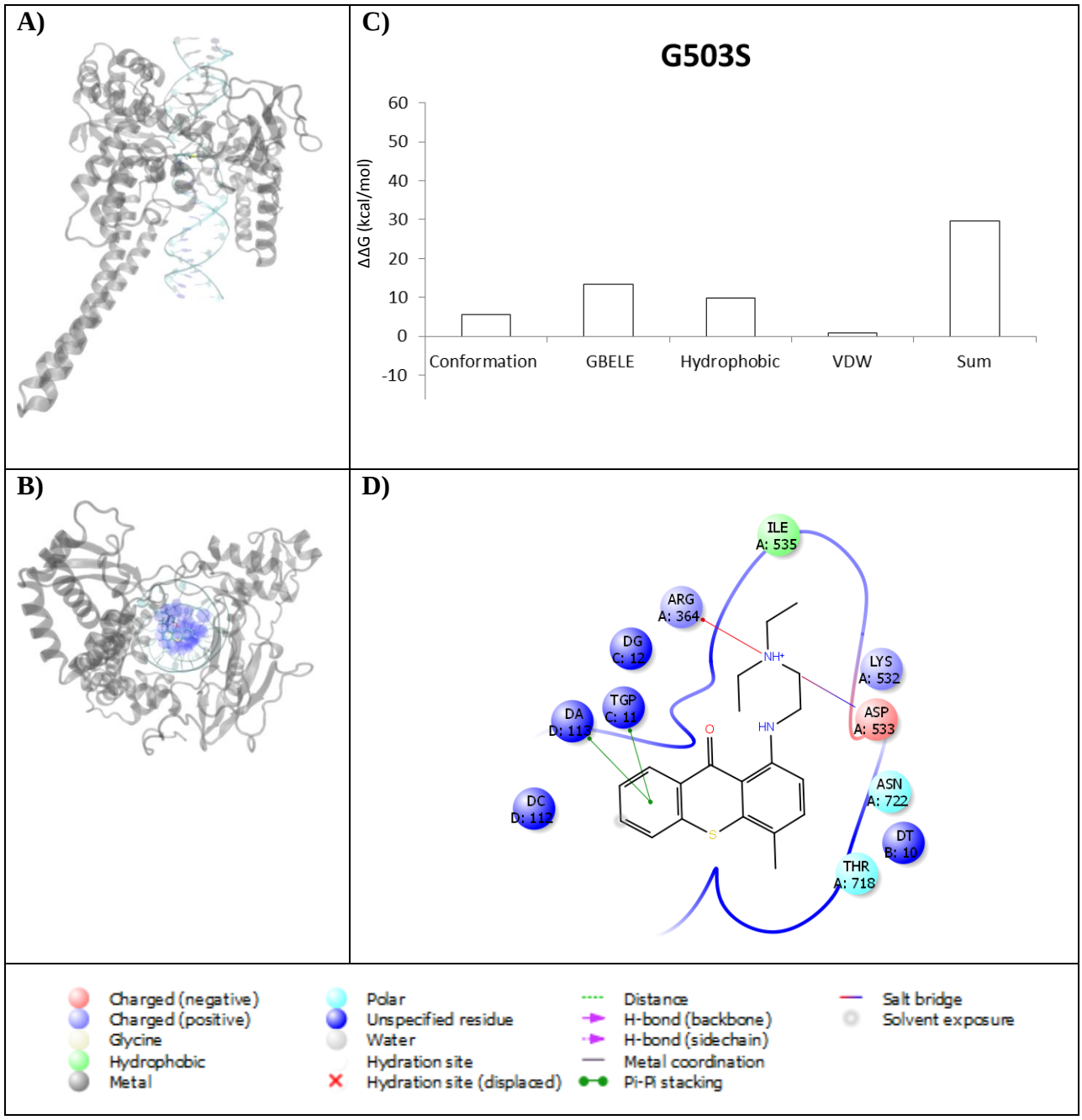


Figure **S80**. Lucanthone Bound to Complex G503S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

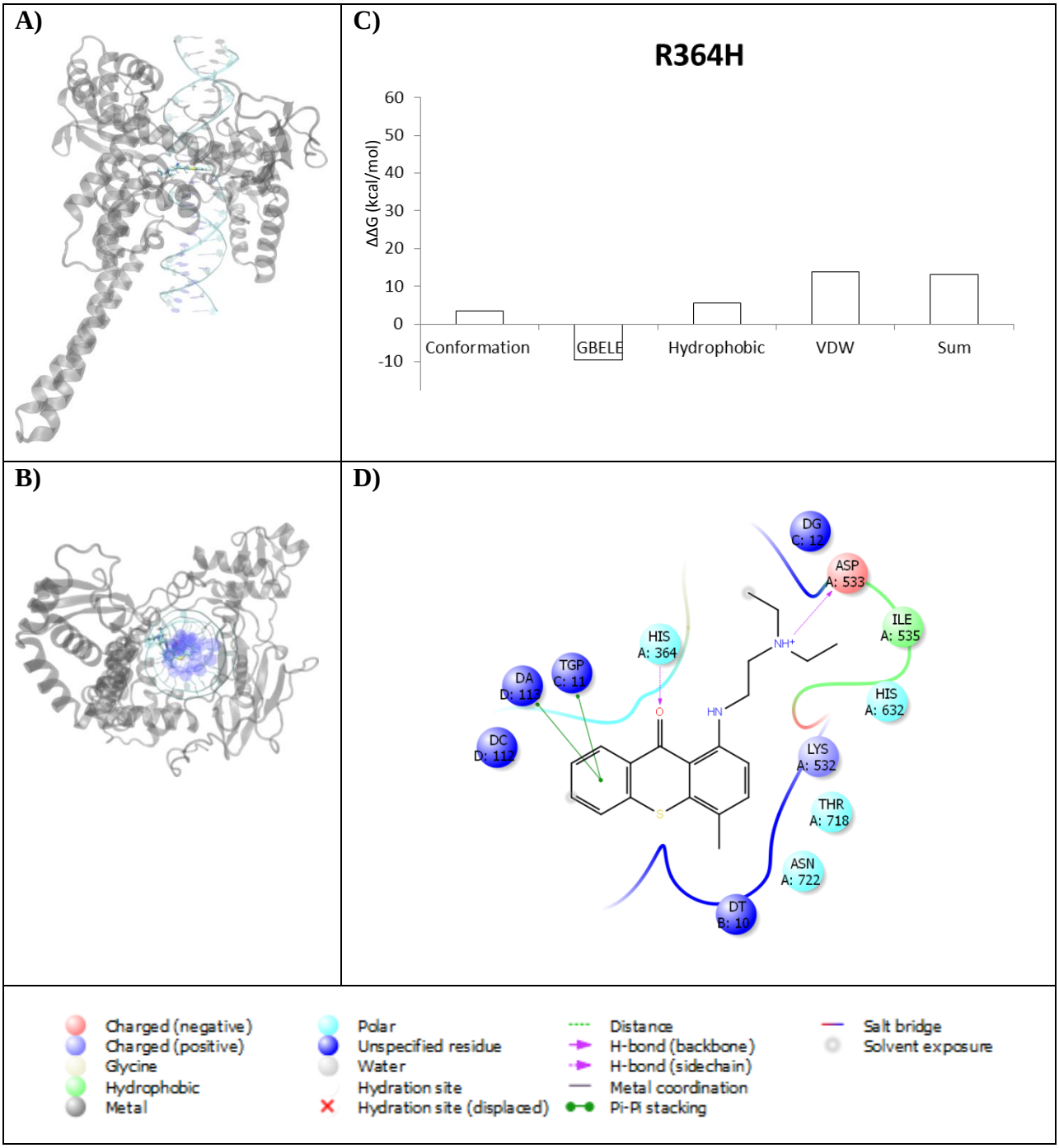


Figure **S81**. Lucanthone Bound to Complex R364H. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

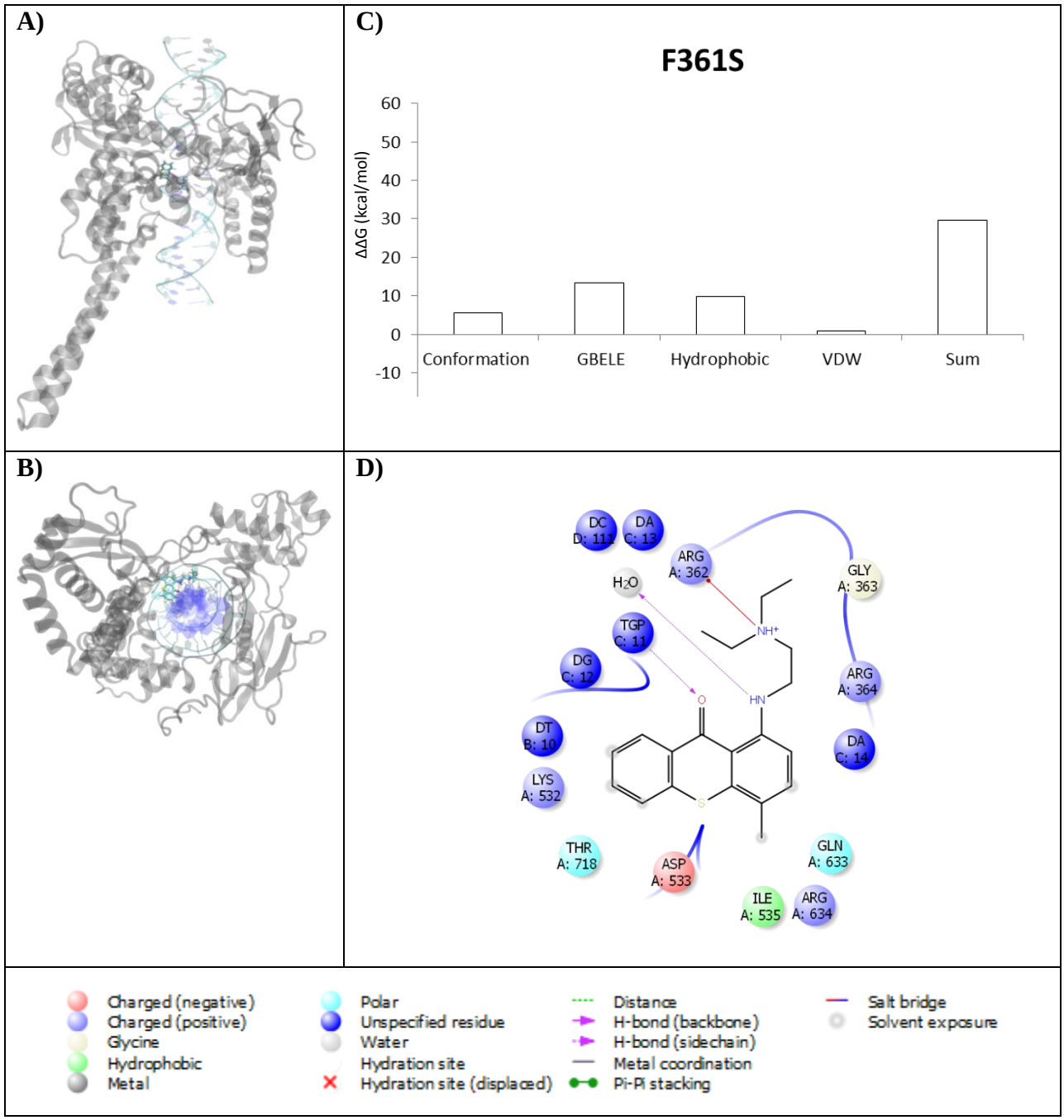


Figure **S82**. Lucanthone Bound to Complex F361S. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

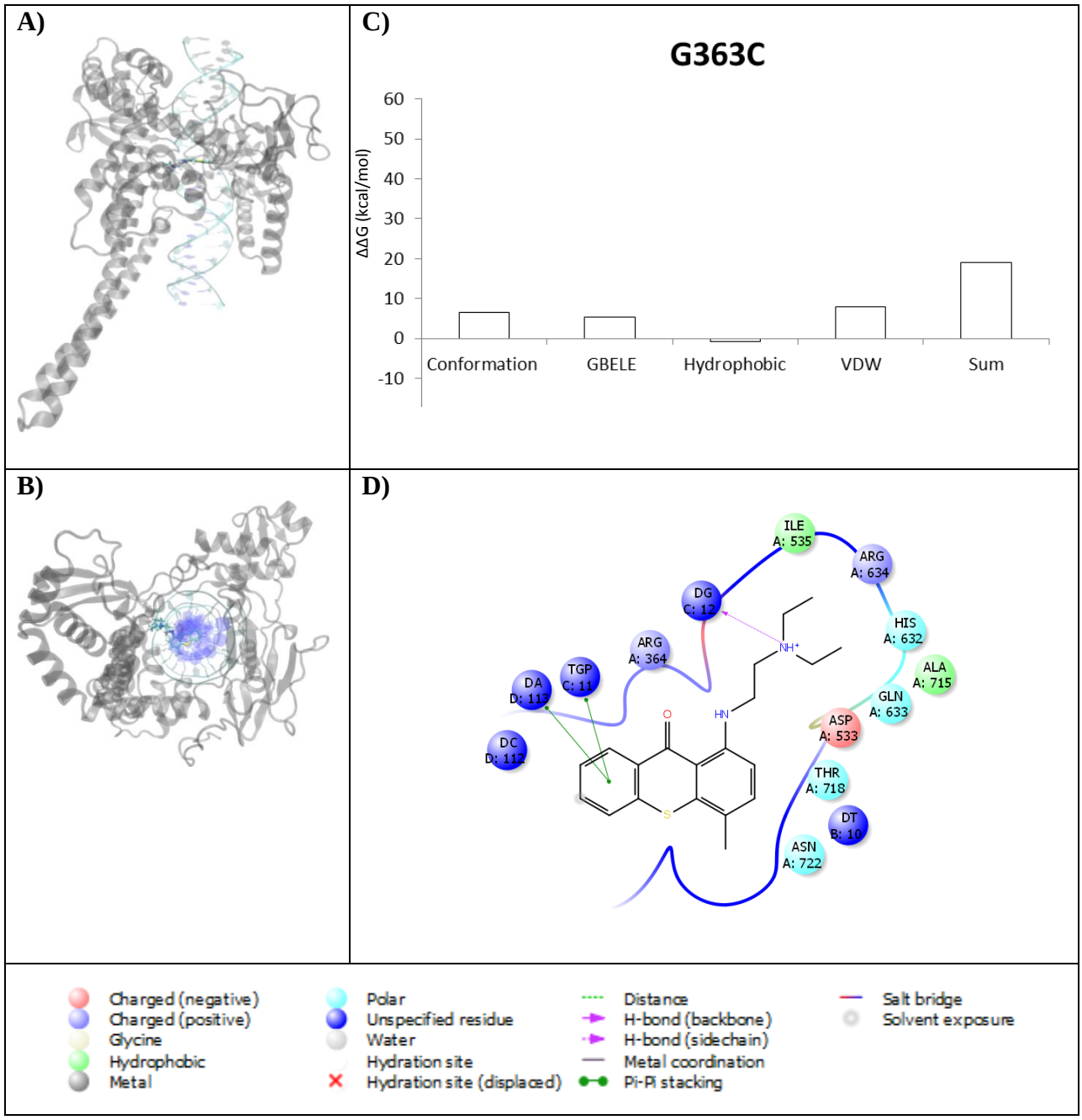


Figure **S83**. Lucanthone Bound to Complex G363C. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex

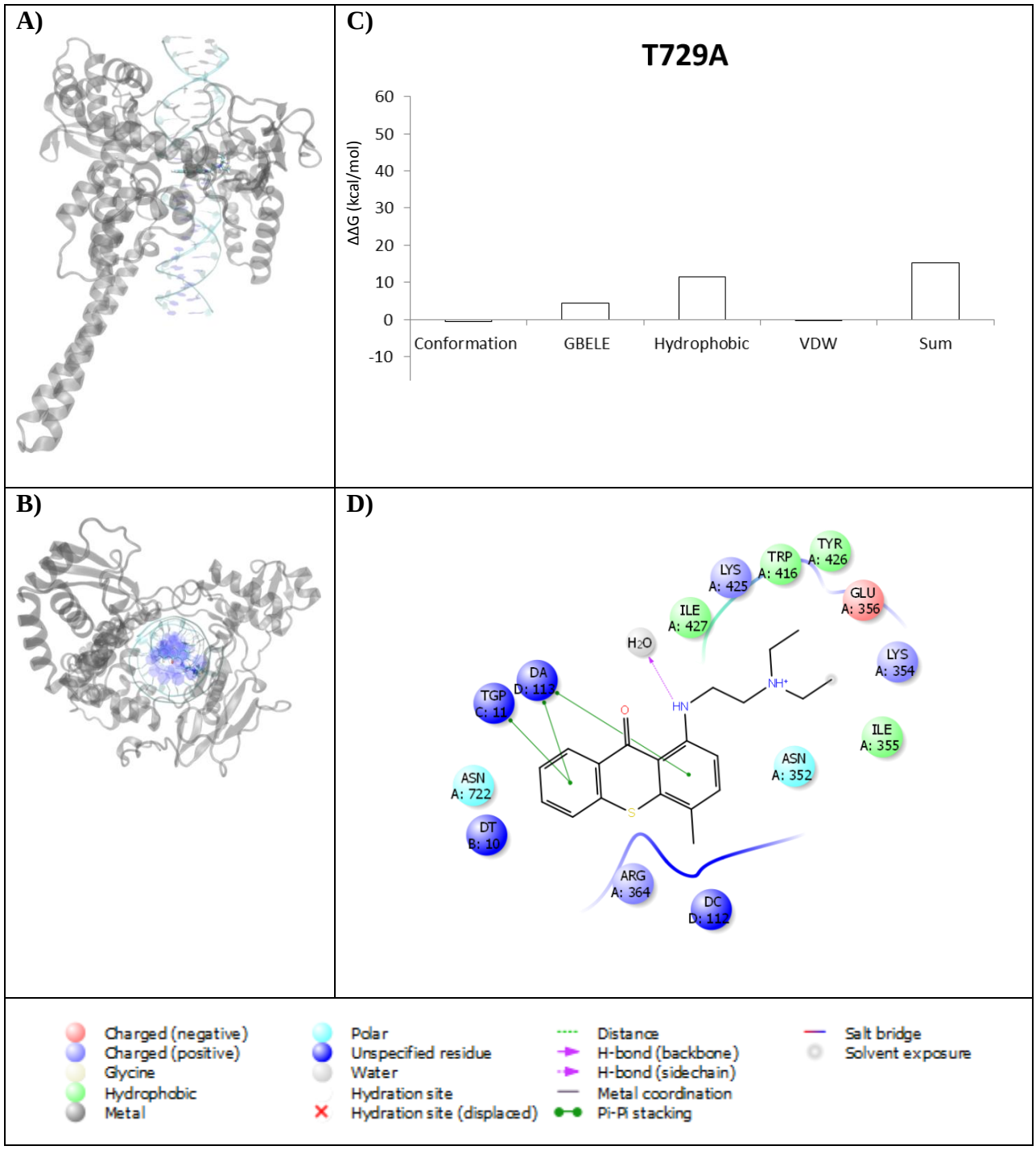
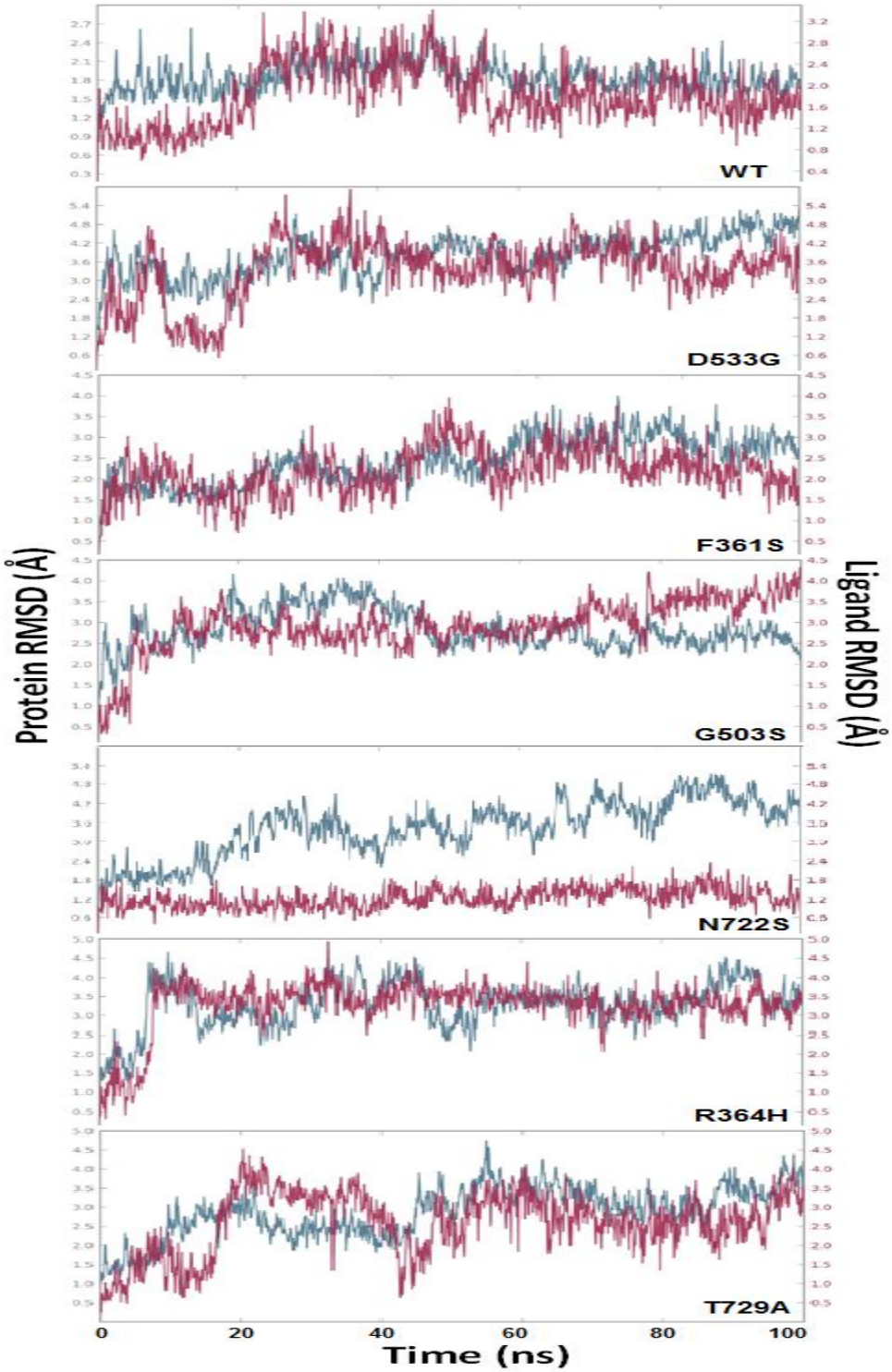
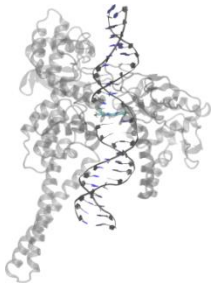
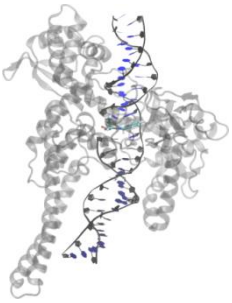
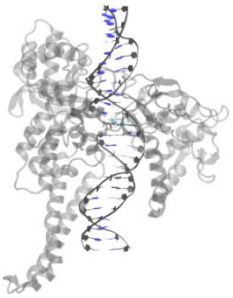
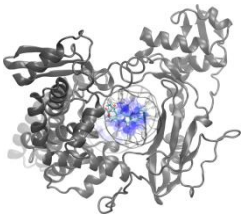
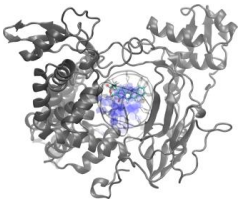
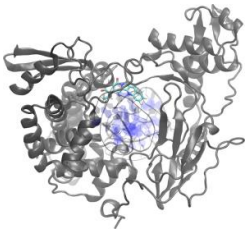
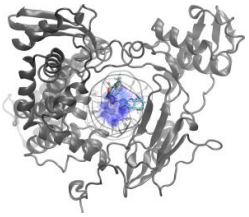


Figure **S84**. Lucanthone Bound to Complex T729A. **A)** 3D top view of complex **B)** 3D side view of complex **C)** Decomposition of ΔΔG **D)** 2D Representation of the binding within the complex



S**85**. Protein RMSD (blue) and Ligand RMSD (red) of topotecan bound to each complex utilizing MD simulations

Cluster ID	WT	D533G	F361S	R364H
Front View				
Top View				
Population	100%	100%	99%	99%

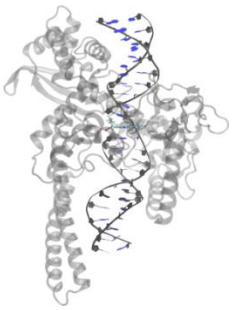
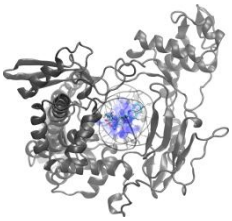
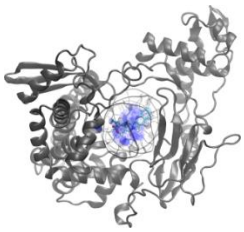
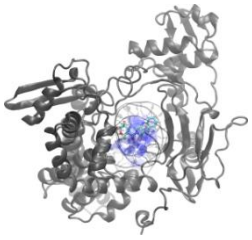
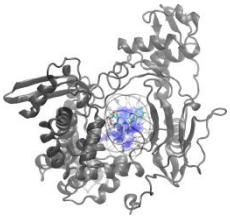
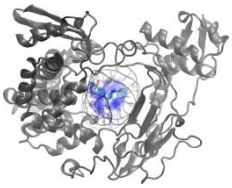
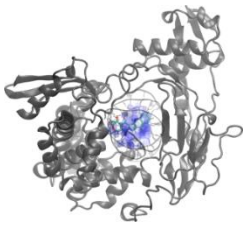
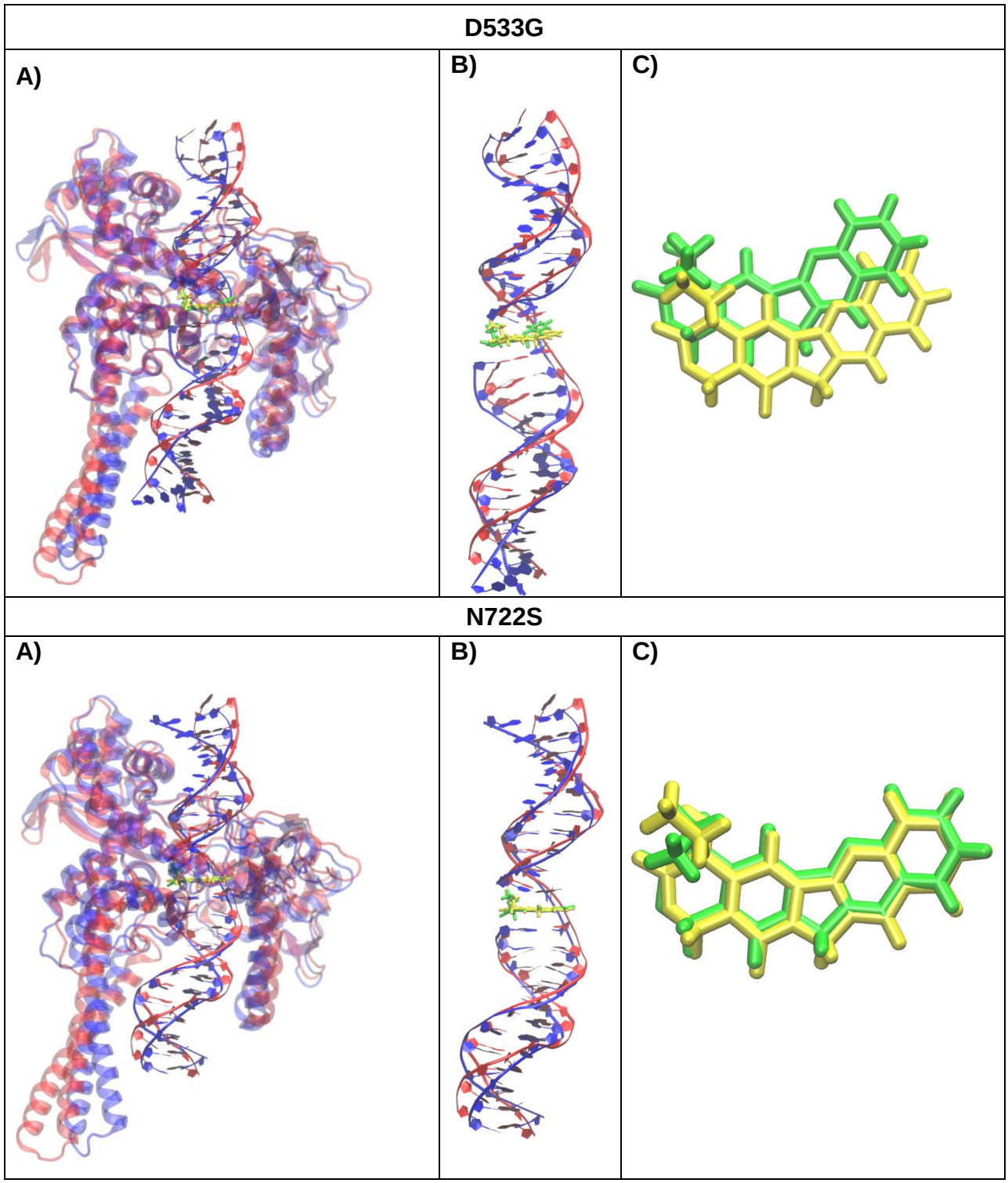
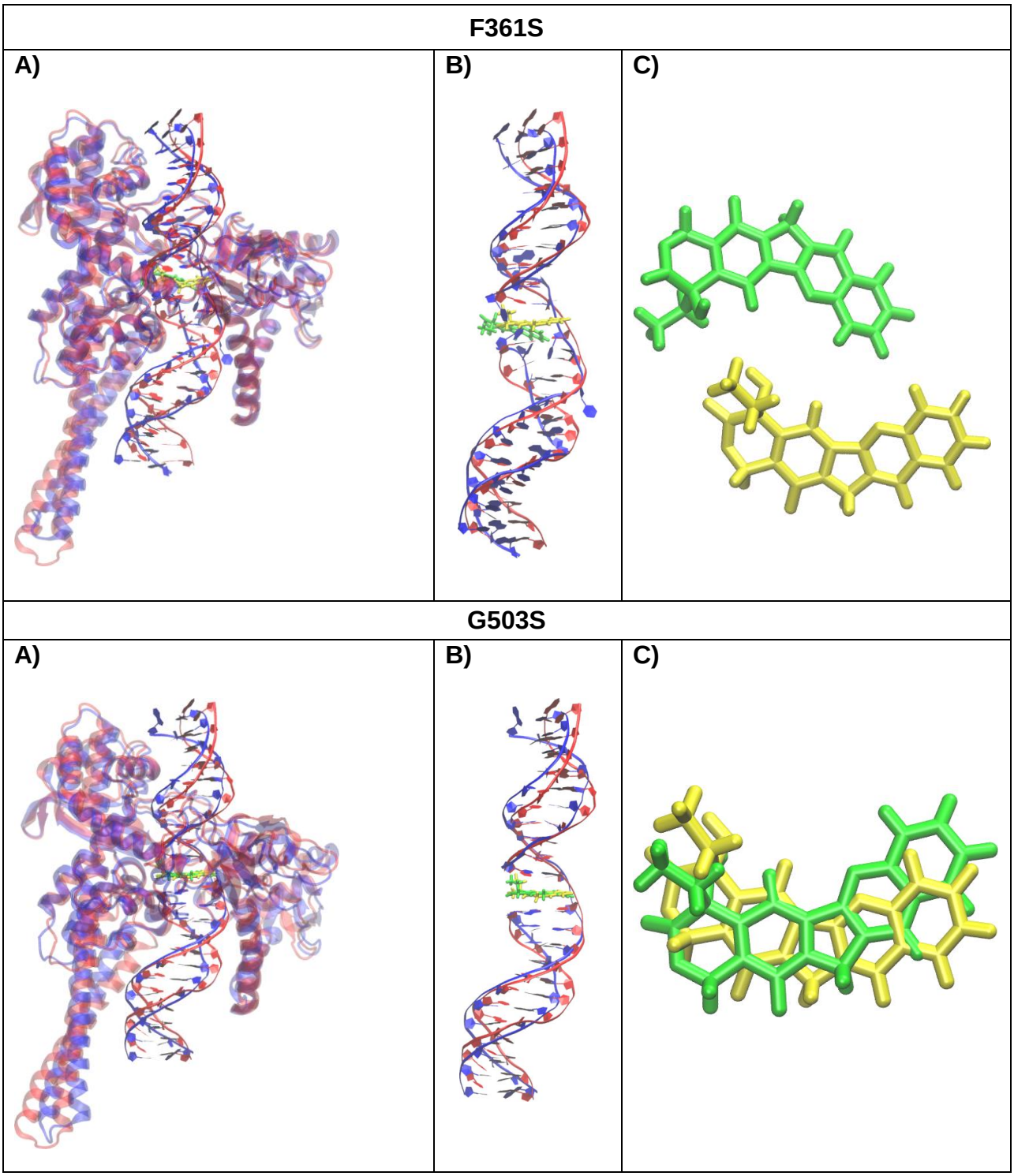
Cluster ID	G503S A	G503S B	N722S A	N722S B
Front View				
Top View				
Population	94%	6.4%	98%	2.3%
Cluster ID	T729A A	T729A B		
Front View				
Top View				
Population	80%	19%		

Figure **S86.** Structures of the most populated complex structure families (population ≥ 2 %) from the clustering analysis of the combined binding trajectories of each topotecan-mutant complex


D533G
A)
B)
C)
N722S
A)
B)
C)


F361S
A)
B)
C)
G503S
A)
B)
C)

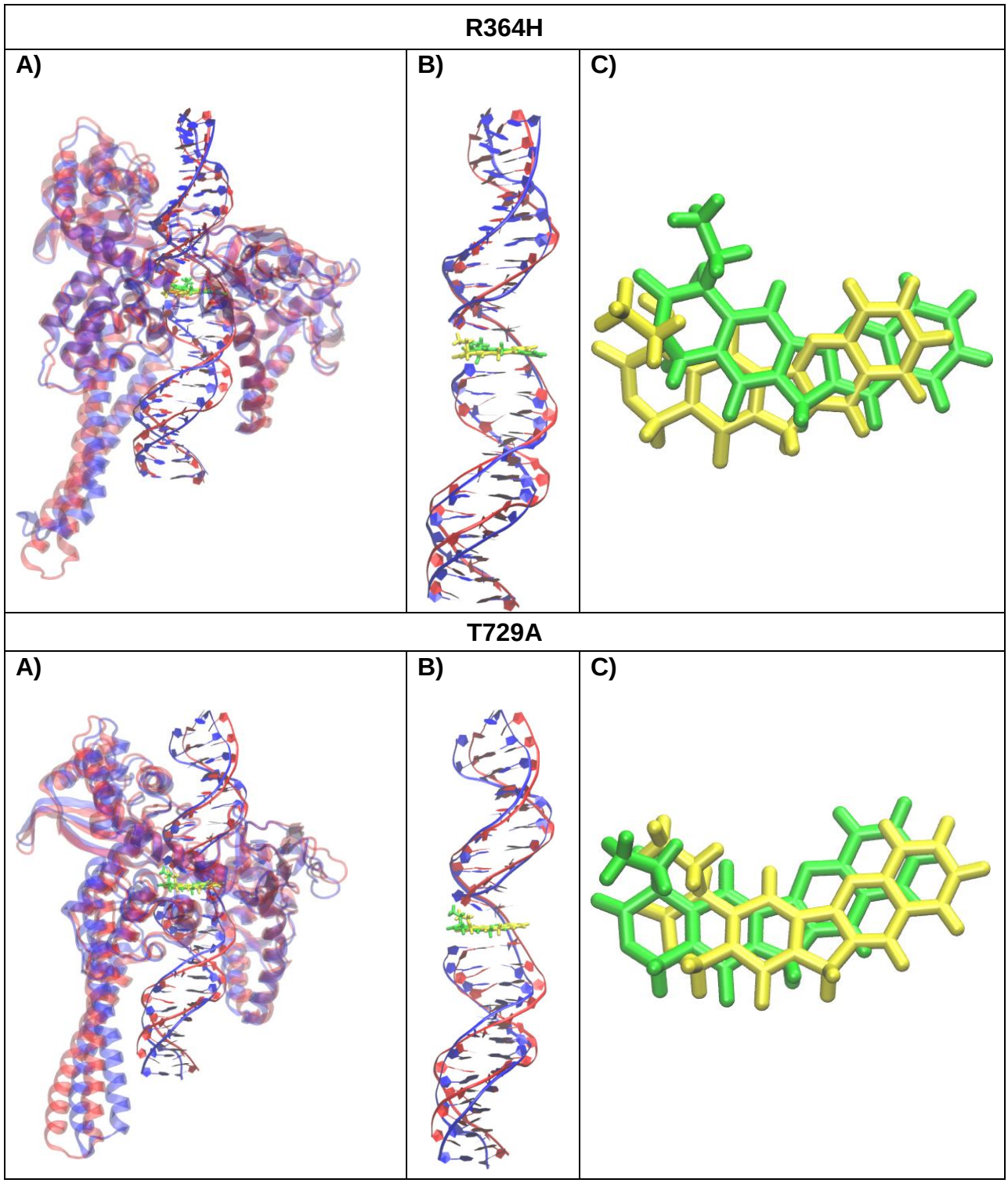


Figure **S87.** Structural comparison of Top1 (**A**), DNA (**B**), and topotecan (**C**) of wild type and mutant complexes as a result of MD simulations. Wild type complex is shown in red and mutant complex in blue. Topotecan bound to wild type is shown in yellow and in green when bound to mutant