Supporting Materials

Structural Refinement of the hERG1 pore and voltage-sensing domain with ROSETTA-TM modeling and Molecular-Dynamics Simulations

Table S1. Used docking parameters for hERG blockers and activators:

(i) AUTODOCK docking program.

Parameters used for blind docking (whole receptor's atoms were used in the mapping of binding site) of ligands	
Parameters	Used value
Number of grid points in each direction	126
Grid spacing	0.75 Å
Grid map dimension in each direction	94.5 Å
Number of hybrid GA-LS runs	200
Grid Center	0.0 0.0 0.0
Parameters used for partitioned docking (focused region of receptor was used in the mapping of binding site) of ligands	
Number of grid points in each direction	126
Grid spacing	0.4 Å
Grid map dimension in each direction	50.4 Å
Number of hybrid GA-LS runs	200
Grid Center	9.00, 8.00,5.00

(ii) GOLD docking program.

Parameters used for blind docking (whole receptor's atoms were used in the mapping of binding site) of ligands	
Parameters	Used value
Maximum length of grid point list	148
Grid spacing	0.75 Å
Calculated cavity radius	83.6 Å

Calculated cavity origin	1.87 -1.71 1.27
Population size	100
Selection pressure	1.1
Number of islands	5
Migrate	10
Mutate	95
Crossover	95
Niche size	2
Number of operation	107000
Parameters used for partitioned docking (focused region of receptor was used in the mapping of binding site) of ligands	
Maximum length of grid point list	90
Grid spacing	0.3 Å
Calculated cavity radius	43.8 Å
Calculated cavity origin	2.37, 3.33, 5.93
Population size	100
Selection pressure	1.1
Number of islands	5
Migrate	10
Mutate	95
Crossover	95
Niche size	2
Number of operation	125000

Table S2. Intra and Inter Subunits Salt-Bridge and Hydrogen-Bonding Statistics* as per atom for S4 voltage sensor.

N^1_{res}	Atom @ N^1_{res}	N^2_{res}	Atom @ N^1_{res}	Inter vs. Intra**	Lifetime (ps)	Occupancy
K525	HN	I521	O	intra	70.8	0.920
	HZ1	E435	OE1	intra	16.1	0.145
			OE2	intra	13.3	0.107
		Q576	OE1	inter	30.0	0.020
	HZ2	E435	OE1	intra	14.7	0.187
			OE2	intra	46.7	0.280
		E575	O	inter	10.0	0.003
		Q576	OE1	inter	16.7	0.017
	HZ3	E435	OE1	intra	11.6	0.147
			OE2	intra	28.0	0.215
		Q576	OE1	inter	10.8	0.022
	O	R528	HN	intra	5.4	0.045
		L529	HN	intra	29.2	0.807
R528	HN	L524	O	intra	82.2	0.932
		K525	O	intra	5.4	0.045
	HE	D460	OD1	intra	80.5	0.563
			OD2	intra	51.3	0.393
	HH11	D456	OD1	intra	53.4	0.890
			OD2	intra	12.9	0.498
			O	intra	5.0	0.007
	HH12	D460	OD1	intra	27.2	0.545
			OD2	intra	41.3	0.688
	HH21	D456	OD1	intra	10.2	0.143
			OD2	intra	14.1	0.385
	O	R531	HN	intra	5.7	0.127
			HE	intra	29.0	0.782
R531	HN	L529	O	intra	5.6	0.120
	HH11	D460	OD1	intra	25.7	0.600
			OD2	intra	39.9	0.745
	HH21	D460	OD1	intra	27.5	0.220
			OD2	intra	29.2	0.302
	HH22	S428	OG	intra	328.3	0.985
	O	R534	HN	intra	19.4	0.732
		V535	HN	intra	5.4	0.022
R534	HN	R531	O	intra	19.4	0.732
	HH11	D466	OD1	intra	36.9	0.528
			OD2	intra	27.8	0.380
		D501	OD1	intra	12.5	0.017
			OD2	intra	5.0	0.002
	HH12	D466	OD1	intra	10.0	0.003
			OD2	intra	30.0	0.010

	HH21	D466	OD1	intra	29.4	0.422
			OD2	intra	44.0	0.367
		D501	OD1	intra	55.0	0.073
			OD2	intra	10.0	0.007
			O	intra	17.5	0.012
	HH22	D501	OD1	intra	268.3	0.268
			OD2	intra	322.5	0.645
			O	intra	15.6	0.047
		A504	O	intra	10.0	0.007
	O	W497	HE	intra	5.0	0.002
		R537	HN	intra	6.9	0.103
		K538	HN	intra	9.4	0.168
R537	HN	V533	O	intra	20.7	0.310
		R534	O	intra	6.9	0.103
		V535	O	intra	5.0	0.007
	HH11	D466	OD1	intra	190.0	0.253
			OD2	intra	228.3	0.457
		N470	OD1	intra	14.8	0.163
	HH12	D501	OD1	intra	87.2	0.582
			OD2	intra	27.3	0.555
	HH21	D466	OD1	intra	21.6	0.438
			OD2	intra	16.8	0.420
		N470	OD1	intra	6.5	0.037
	HH22	K495	O	intra	5.0	0.002
	O	L539	HN	intra	6.7	0.007
		D540	HN	intra	12.8	0.362
K538	HN	V535	O	intra	9.4	0.168
		L539	O	intra	11.8	0.358
	O	D540	NH	intra	5.4	0.023

*The production run of 3000 ps have been used to average interactions between amino-acid residues every 5 ps.

** Interaction within same chain is marked as “intra” and between two different chains “inter”

Table S3. Comparison of docking poses populations of ligands at EC, IC and outer mouth of selectivity filter (SF) of hERG; their close contacts with target and binding scores of top poses at focused region derived by AUTODOCK.

	IC (%)	Outer mouth of SF (%)	EC (%)
Dofetilide	93	5	2

	IC (%)	Outer mouth of SF (%)	EC (%)
KN-93	58	38	4

NFA	IC (%)	Outer mouth of SF (%)	EC (%)
NFA (neutral)	6	28	66
NFA (anion)	12	17	71

Dofetilide	**Close contacts**
IC site	Leu622, Thr623, Ser624, Val625, Gly626, Ser649, Tyr652, Phe656

KN-93	**Close contacts**
IC site	Leu622, Thr623, Ser624, Val625, Gly626, Ser649, Tyr652, Phe656
Outer mouth of SF	Ser600, Gly601, Asn629, Gly628, Ser631, Phe627

NFA (anion)	**Close contacts**
EC site	Glu438, Glu437, Lys434, Thr436, Met574, Lys525, Leu433, Glu435, Lys595, Lys610
Outer mouth of SF	Leu602, Asn598, Ser600, Tyr597, Ser599, Asn629, Val630, Ser631, Gly601, Tyr616, Pro632, Asn633, Thr634

NFA (neutral)	**Close contacts**
EC site	Glu438, Glu437, Lys434, Thr436, Lys525, Met574, Leu433, Glu435, Lys595, Lys610
Outer mouth of SF	Asn633, Asn629, Ser600, Tyr616, Leu602, Glu637, Thr634, Lys638, Asn635, Asn629, Tyr597, Asn598, Ser599

Ligands	**Binding Score (kcal/mol)**
Dofetilide	-8.26
KN-93	-9.22
NFA (neutr.)	-7.36
NFA (anion)	-6.33

Table S4. Comparison of docking poses populations of ligands at EC, IC and outer mouth of selectivity filter (SF) of hERG; their close contacts with target and binding scores of top poses at focused region derived by GOLD.

	IC (%)	Outer mouth of SF (%)	EC (%)
Dofetilide	94	6	-

	IC (%)	Outer mouth of SF (%)	EC (%)
KN-93	86	14	-

NFA	IC (%)	Outer mouth of SF (%)	EC (%)
NFA (neutral)	-	38	62
NFA (anion)	-	23	77

Dofetilide	**Close contacts**
IC site	Thr623, Ser624, Ser649, Tyr652, Ala653, Phe656

KN-93	**Close contacts**
IC site	Thr623, Ser624, Met645, Ser649, Tyr652, Ala653, Phe656, Gly657

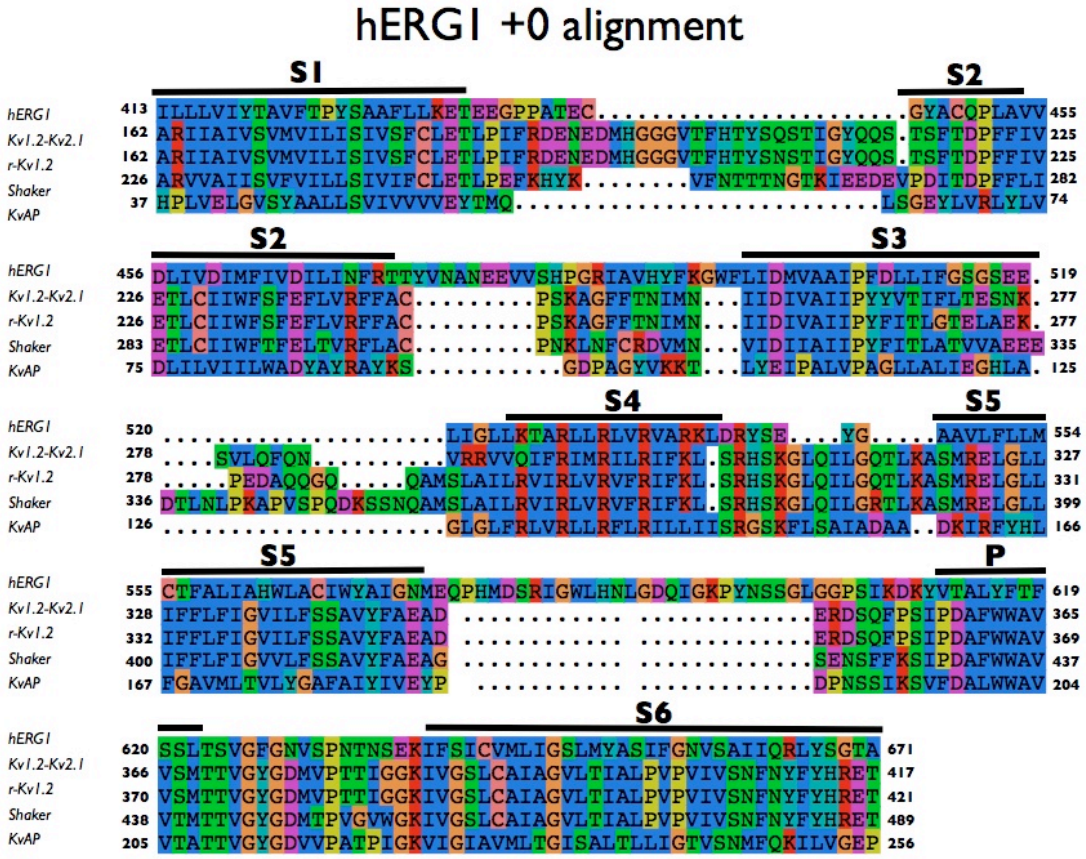
NFA (anion)	**Close contacts**
EC site	Thr436, Lys595, Tyr569, Ala570, Asn573, Lys610, Thr613, Tyr611, Met574
Outer mouth of SF	Asn629, Ser600, Tyr597, Asn598, Gly628, Ser631

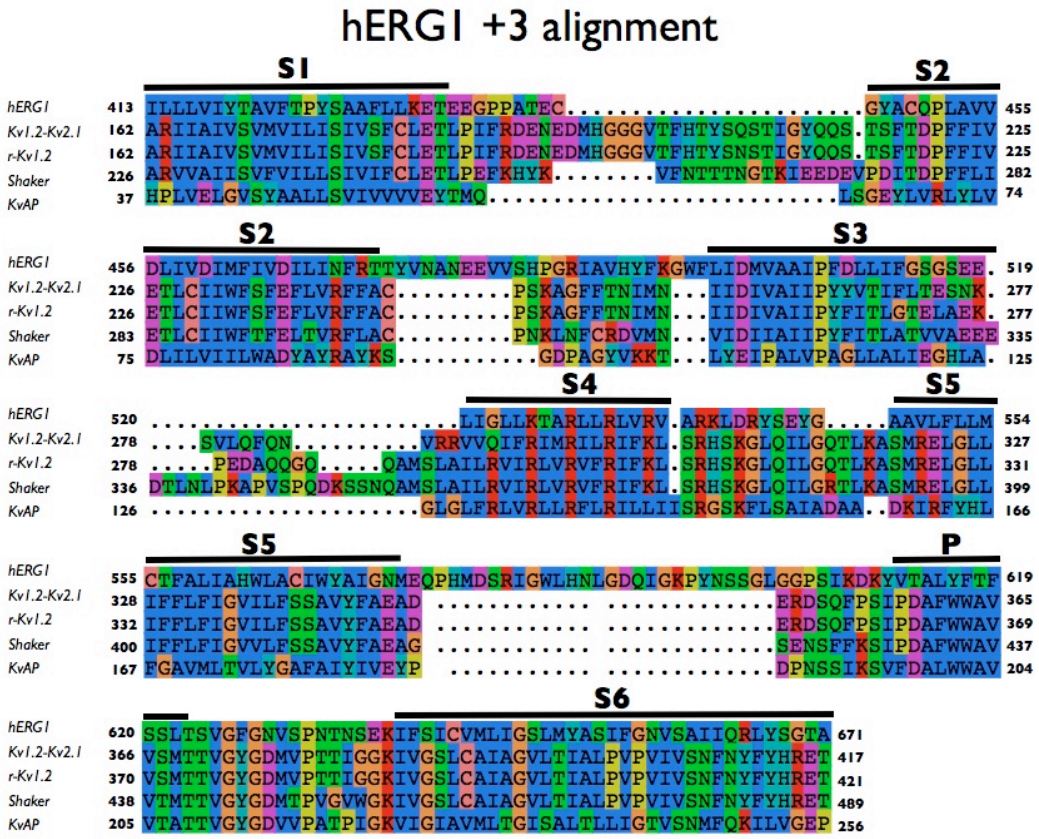
NFA (neutral)	**Close contacts**
EC site	Lys595, Asn573, Asn598, Met574, Ala570, Leu602, Thr613, Tyr611, Lys610
Outer mouth of SF	Tyr597, Gly628, Asn629, Ser600, Phe627, Asn598, Ser599, Ser631

Ligands	**GOLD/ChemScore Binding Score (kcal/mol)**	**GOLD Fitness Score**
Dofetilide	-8.07	63.59
KN-93	-8.48	67.85
NFA (neutr.)	-6.41	47.17
NFA (anion)	-6.37	43.85

Figure S1. Alignment used for homology models. Several alignments for S4 helix have been considered to build models with Rosetta modeling and MD simulations. (i) hERG1 +0 alignment; (ii) hERG1 +3 alignment.

(i)




hERG1 +3 alignment
S1
S2
S3
S4
S5
P
S6
hERG1 413 ILLLVIYTAVFTPYSAAFLLKETEEGPPATEC.....GYACQPLAVV 455
Kv1.2-Kv2.1 162 ARIIAIVSVMVILISIVSFCLETLPIFRDENEDMHGGGVTFHTYSQSTIGYQQS.TSFTDPFFIV 225
r-Kv1.2 162 ARIIAIVSVMVILISIVSFCLETLPIFRDENEDMHGGGVTFHTYSNSTIGYQQS.TSFTDPFFIV 225
Shaker 226 ARVVAIISVFVILLSIVIFCLETLPEFKHYK.....VFNTTTNGTKIEEDEVPDITDPFFLI 282
KvAP 37 HPLVELGVSYAALLSVIVVVVEYTMQ.....LSGEYLVRLYLV 74
hERG1 456 DLIVDIMFIVDILINFRTTYVNANEEVVSHPGRIAVHYFKGWFLIDMVAAIPFDLLIFGSGSEE. 519
Kv1.2-Kv2.1 226 ETLCIIWFSFEFLVRFFAC.....PSKAGFFTNIMN...IIDIVAIIPYYVTIFLTESNK. 277
r-Kv1.2 226 ETLCIIWFSFEFLVRFFAC.....PSKAGFFTNIMN...IIDIVAIIPYFITLGTELAEK. 277
Shaker 283 ETLCIIWFTFELTVRFLAC.....PNKLNFCRDVMN...VIDIIAIIPYFITLATVVAEEE 335
KvAP 75 DLILVIILWADYAYRAYKS.....GDPAGYVKKT...LYEIPALVPAGLLALIEGHLA. 125
hERG1 520 .....LIGLLKTARLLRLVRV.ARKLDRYSEYG.....AAVLFLLM 554
Kv1.2-Kv2.1 278 ....SVLQFQN.....VRRVVQIFRIMRILRIFKL.SRHSKGLQILGQTLKASMRELGLL 327
r-Kv1.2 278 .....PEDAQQGQ.....QAMSLAILRVIRLVRVFRIFKL.SRHSKGLQILGQTLKASMRELGLL 331
Shaker 336 DTLNLPKAPVSPQDKSSNQAMSLAILRVIRLVRVFRIFKL.SRHSKGLQILGRTLKASMRELGLL 399
KvAP 126 .....GLGLFRLVRLLRFLRILLIISRGSKFLSAIADAA..DKIRFYHL 166
hERG1 555 CTFALIAHWLACIWYAIGNMEQPHMDSRIGWLHNLGDQIGKPYNSSGLGGPSIKDKYVTALYFTF 619
Kv1.2-Kv2.1 328 IFFLFIGVILFSSAVYFAEAD ....ERDSQFPSIPDAFWWAV 365
r-Kv1.2 332 IFFLFIGVILFSSAVYFAEAD ....ERDSQFPSIPDAFWWAV 369
Shaker 400 IFFLFIGVVLFSSAVYFAEAG ....SENSFFKSIPDAFWWAV 437
KvAP 167 FGAVMLTVLYGAFAIYIVEYP ....DPNSSIKSVFDALWWAV 204
hERG1 620 SSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVSAIIQRLYSGTA 671
Kv1.2-Kv2.1 366 VSMTTVGYGDMVPTTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRET 417
r-Kv1.2 370 VSMTTVGYGDMVPTTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRET 421
Shaker 438 VTMTTVGYGDMTPVGVWGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRET 489
KvAP 205 VTATTVGYGDVVPATPIGKVIGIAVMLTGISALTLLIGTVSNMFQKILVGEP 256

(ii)

Figure S2. Representation of partitioned docking (the channel was mapped using a cross-section of one of four VS domains together with the pore domain region (highlighted regions at the Figure, top view)).

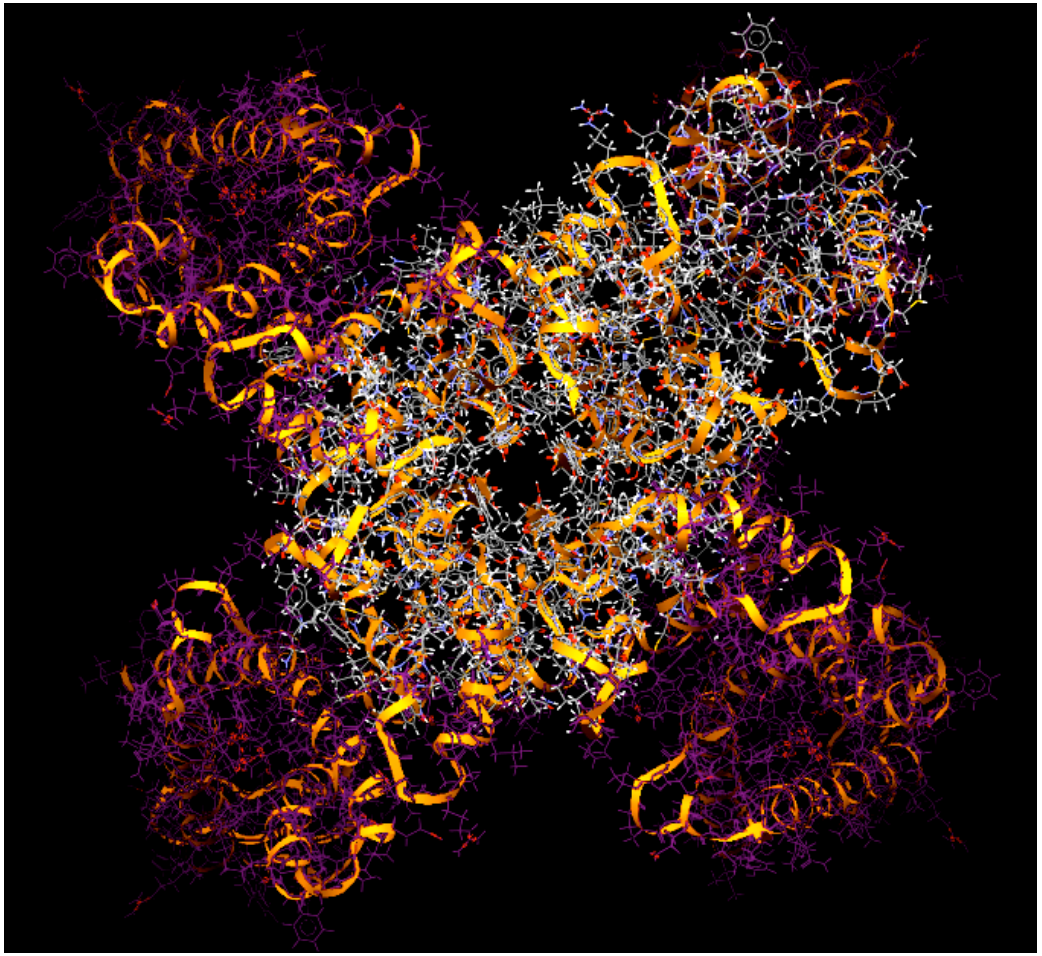
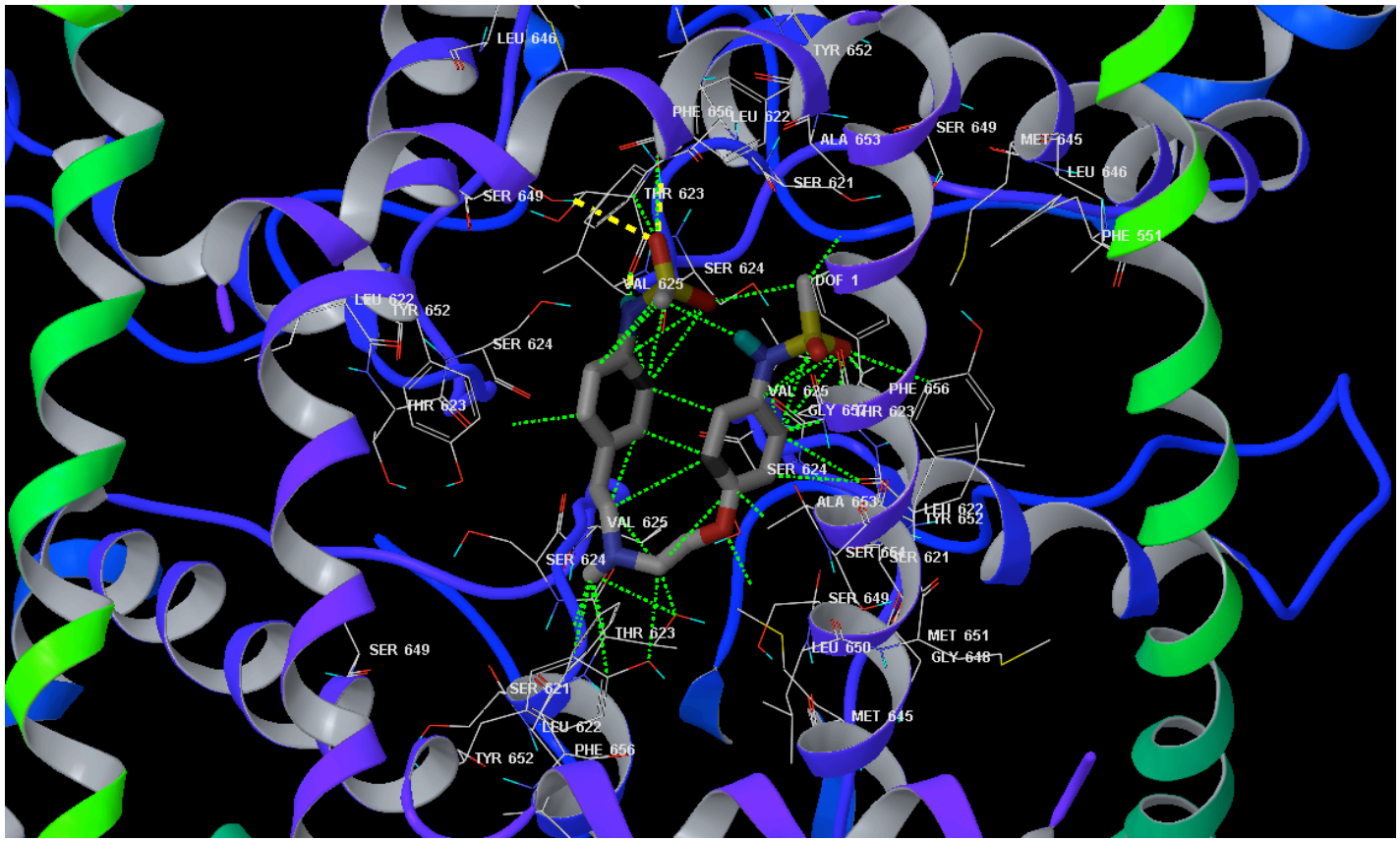


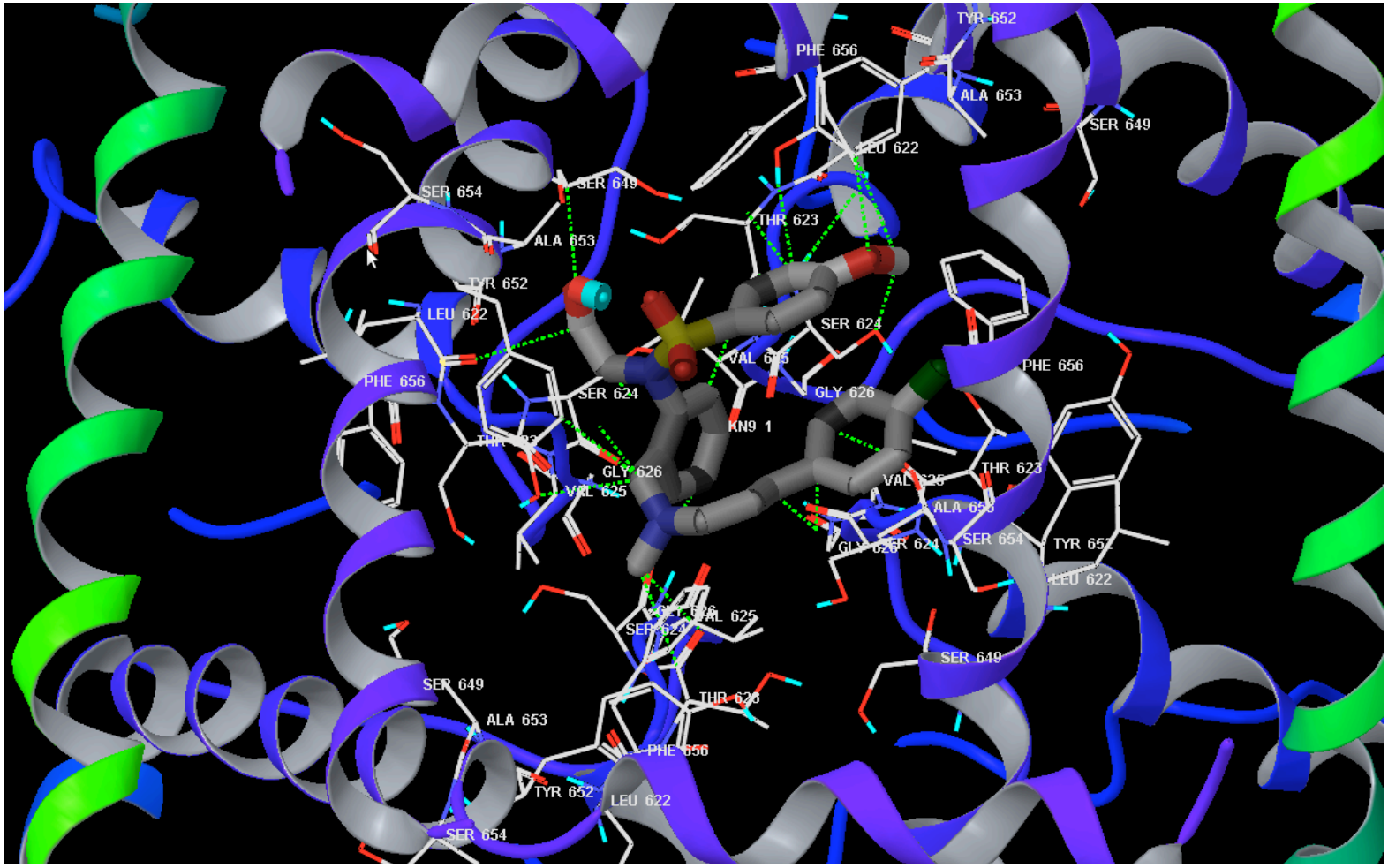
Figure S3. Binding interactions of ligands at hERG. Yellow and green dashed bonds show H-bonds and close-van der Waals contacts, respectively.

(i) Top docking poses derived by AUTODOCK

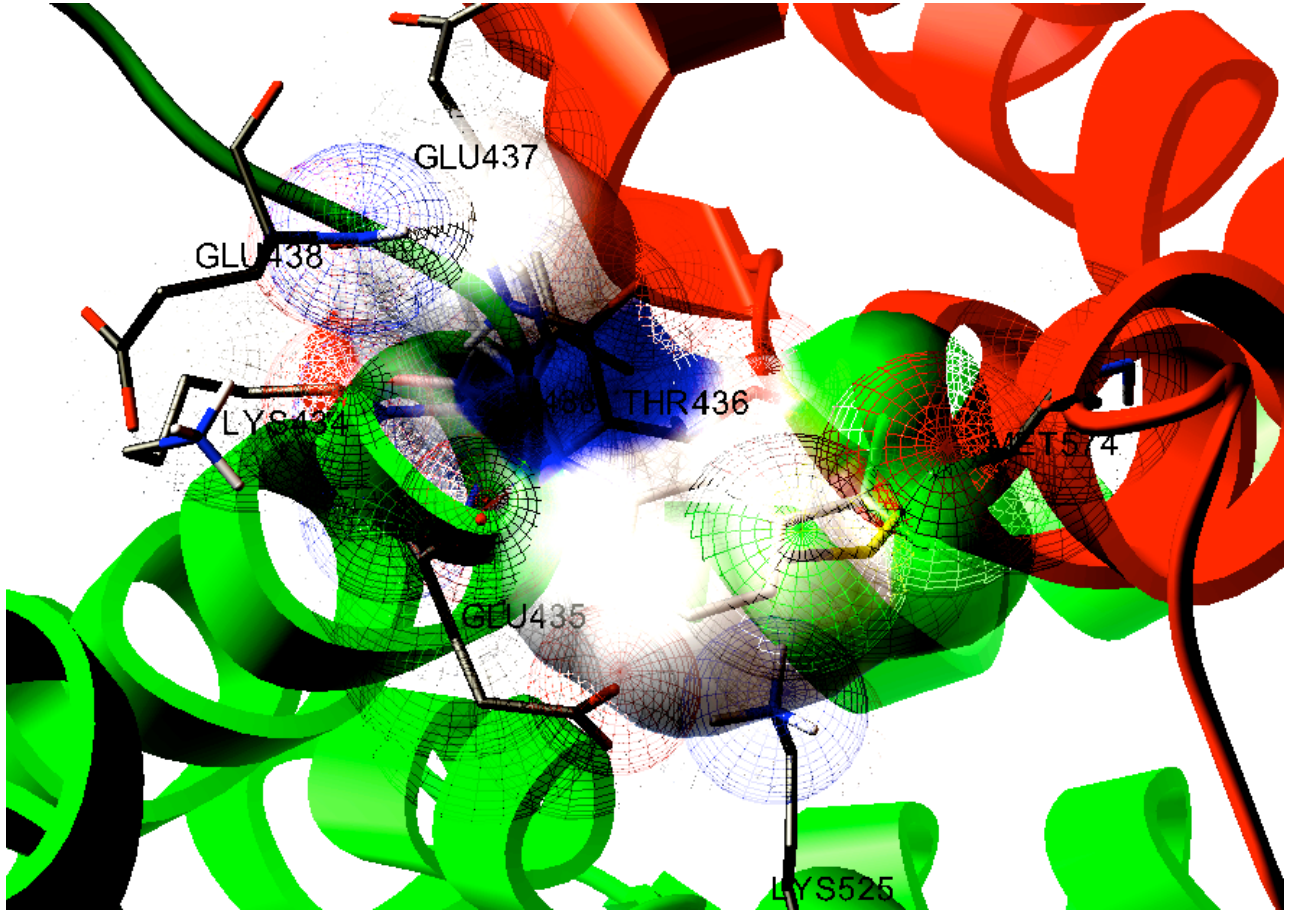
Dofetilide at IC (top view)



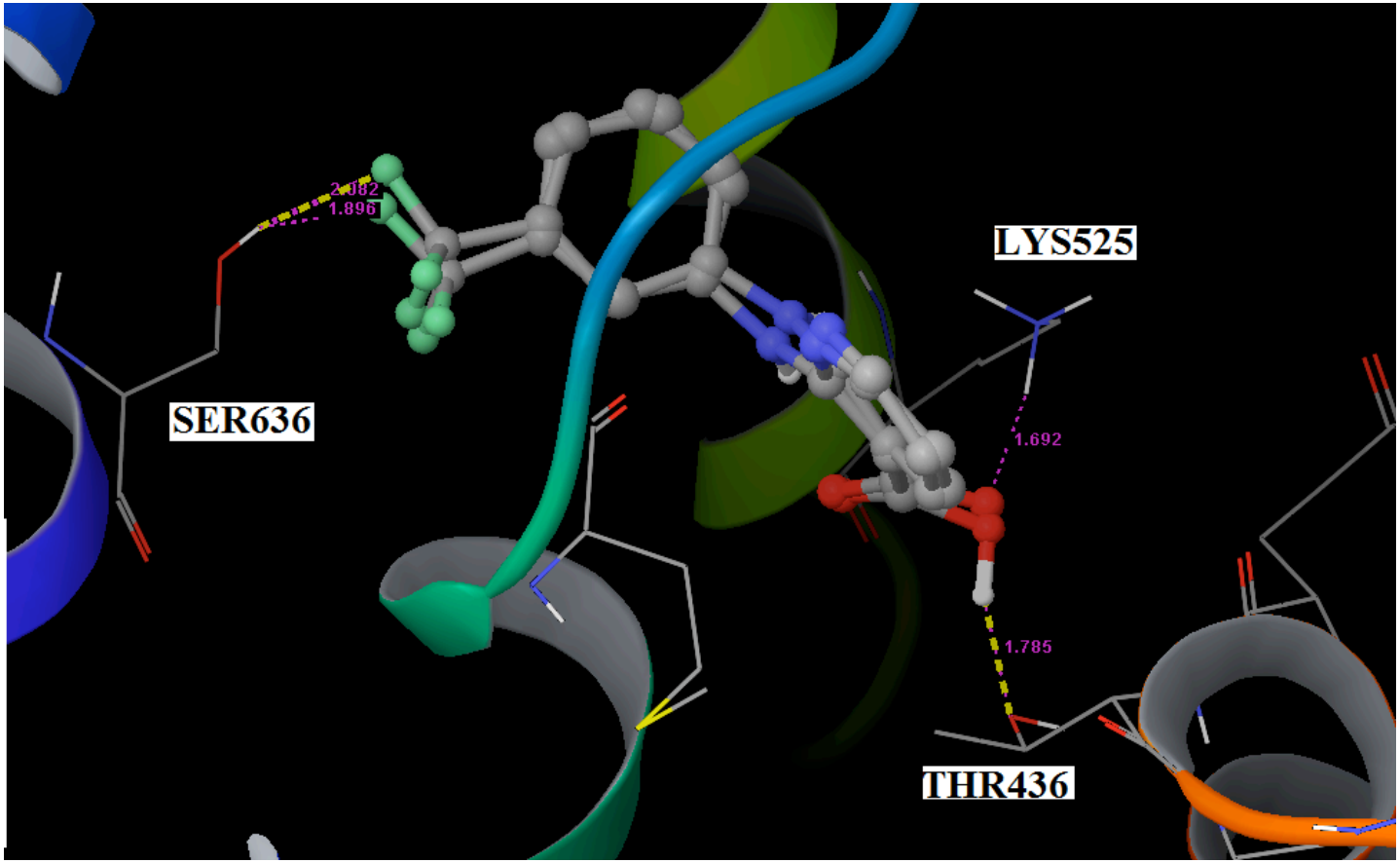
KN93 at IC (top view)



NFA at EC (top view)

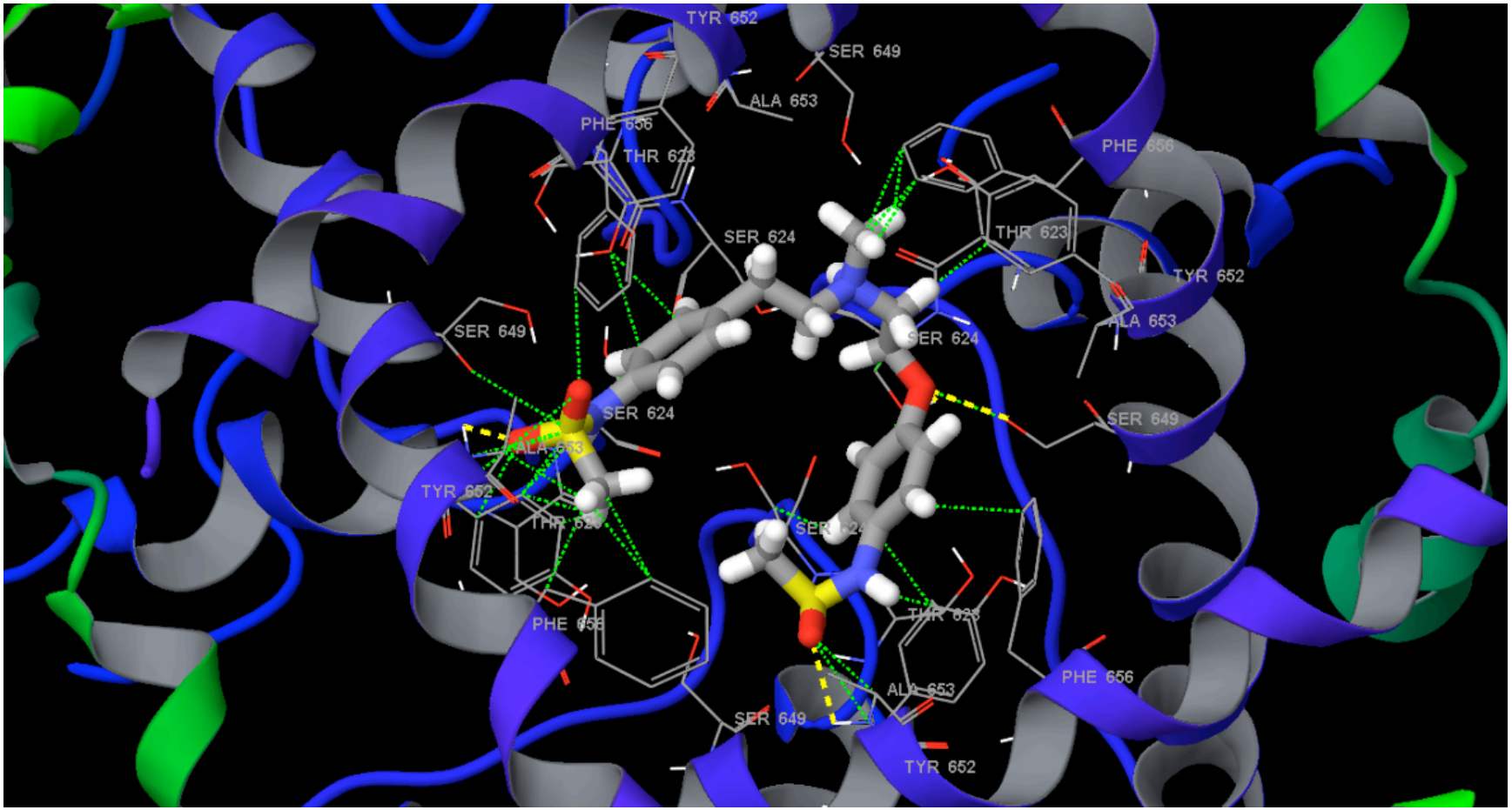


NFA (superimposition of its neutral and anionic forms) at EC

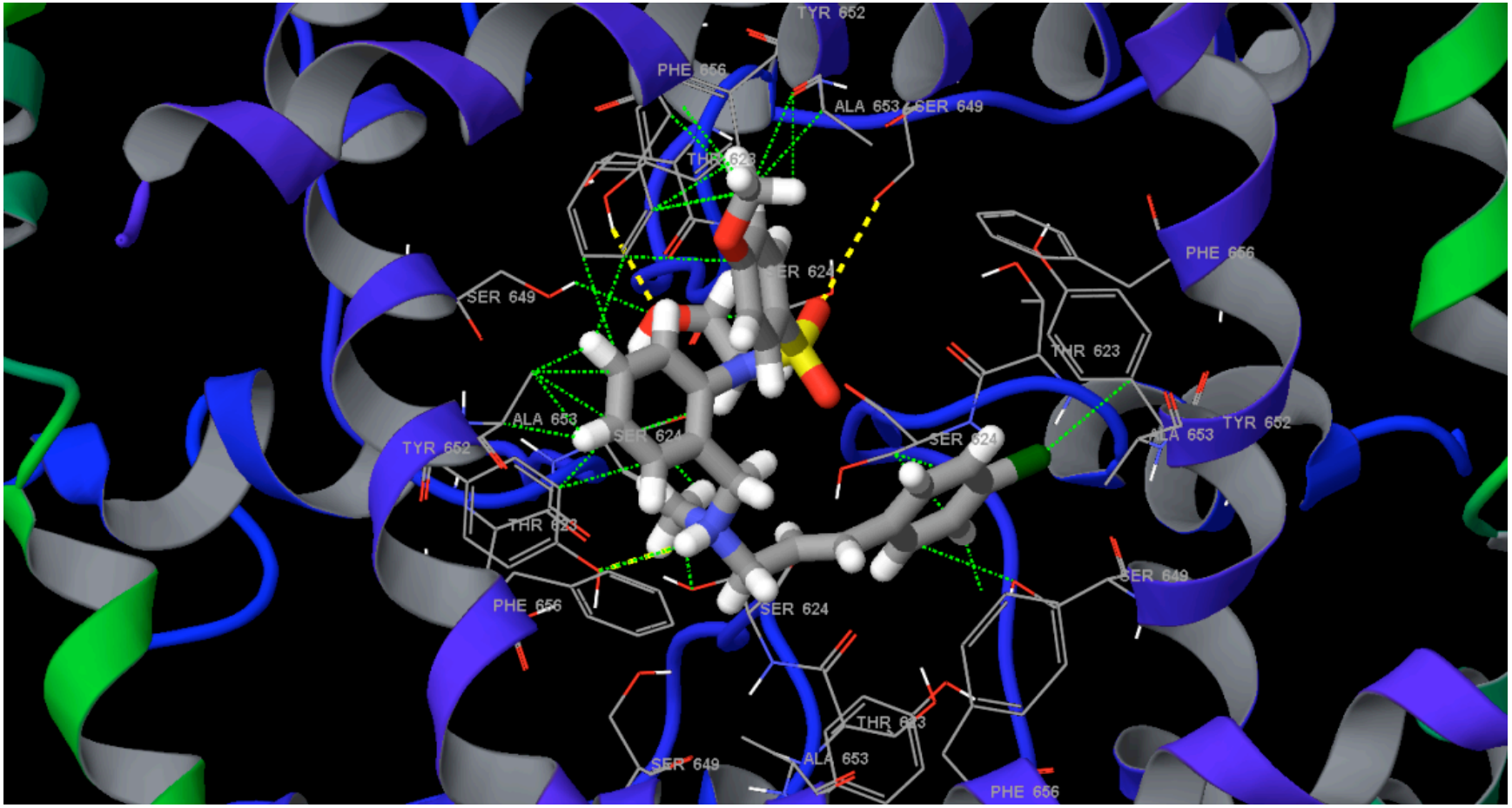


(ii) Top docking poses derived by GOLD

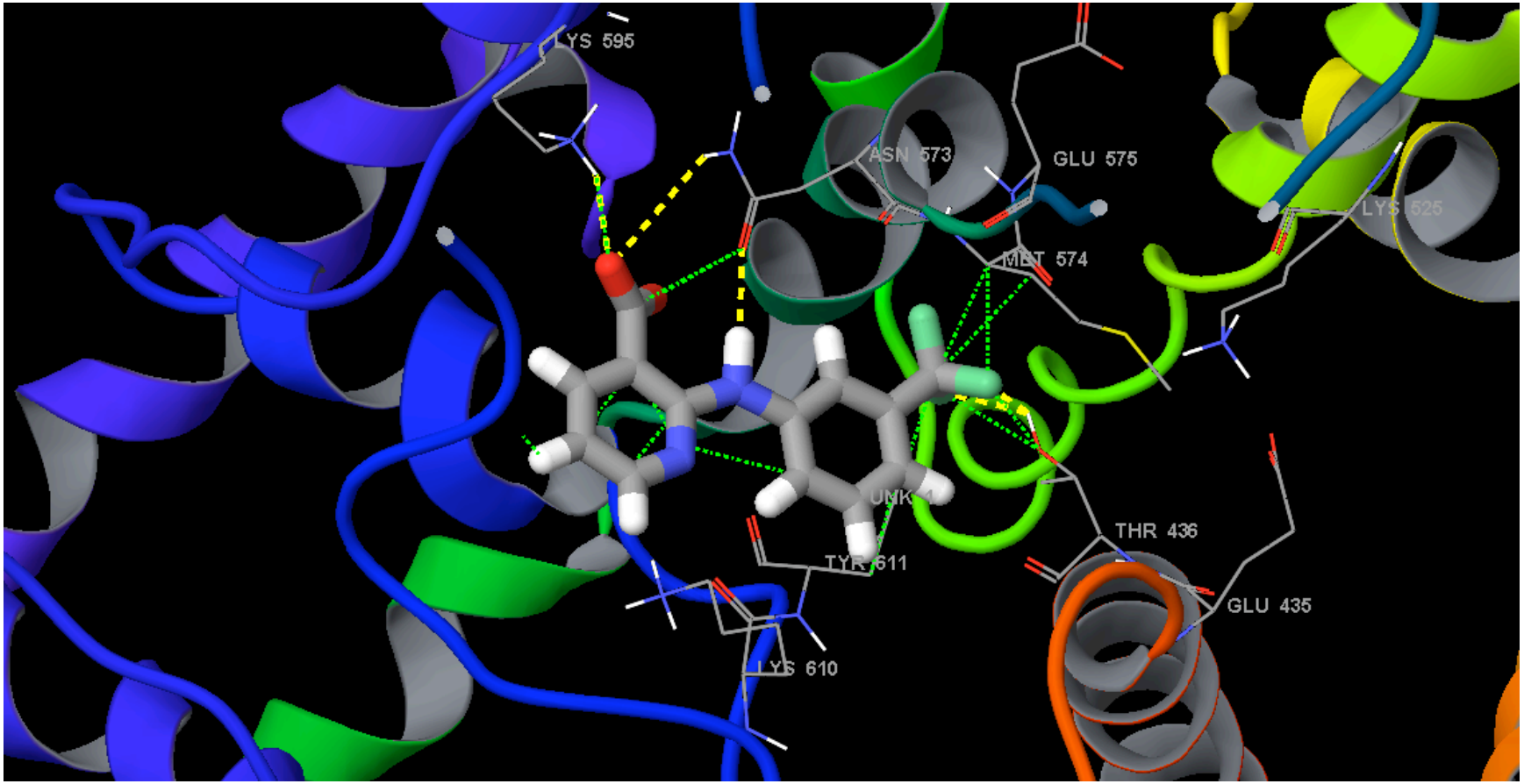
Dofetilide at IC (top view)



KN93 at IC (top view)



NFA at EC (top view)



NFA (superimposition of its neutral (shown in sticks drawing) and anionic (shown in ball and sticks drawing) forms) at EC