

Structural Insights and Influence of V599 Mutations on the Overall Dynamics of *BRAF* Protein against its Kinase Domains

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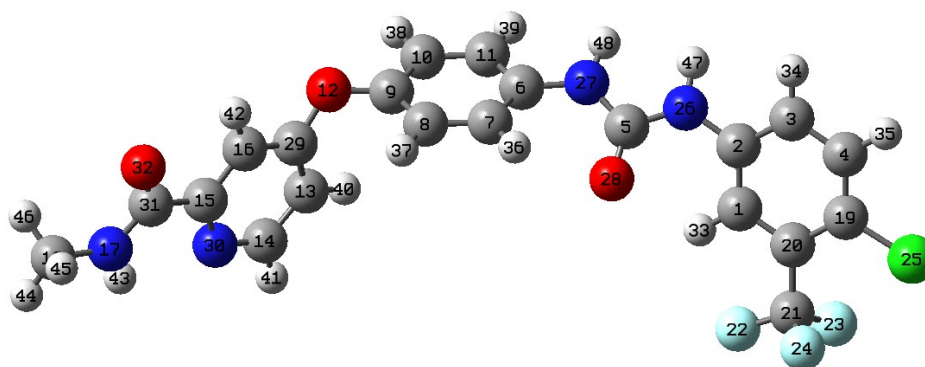
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Table ST1 Structural parameters of optimized BAY43-9006 molecule obtained from Gaussian studies.



Tag	Symbol	Bond	Angle	Dihedral	Tag	Symbol	Bond	Angle	Dihedral
1	C				25	Cl	1.729972	118.7175	-179.651
2	C	1.414924			26	N	1.342625	27.61832	-153.391
3	C	1.409376	118.7801		27	N	1.33991	140.914	-145.192
4	C	1.408925	120.2578	-1.03409	28	O	1.230692	95.3418	31.86152
5	C	2.47194	99.65163	-167.991	29	C	1.373633	120.3064	-57.8315
6	C	2.465769	168.6069	-143.522	30	N	1.350269	123.3867	-5.32179
7	C	1.416376	100.372	-152.975	31	C	1.334292	33.90364	174.5263
8	C	1.406501	120.4667	-167.056	32	O	1.233285	122.1671	178.1976
9	C	1.407936	120.3363	-0.06129	33	H	1.078905	118.4848	179.2228
10	C	1.408783	119.4187	-0.03517	34	H	1.079752	120.5378	-179.219
11	C	1.409302	120.4176	-0.34514	35	H	1.08019	119.8348	-179.011
12	O	1.370613	121.2016	-175.753	36	H	1.079668	121.2391	13.61509
13	C	2.443554	99.32724	-78.9205	37	H	1.077026	119.5897	-179.229
14	C	1.403142	146.42	151.2122	38	H	1.080643	119.8944	179.6567
15	C	2.322543	92.34131	-6.74586	39	H	1.080466	118.8772	179.827
16	C	1.408944	91.02484	-0.26874	40	H	1.079459	92.89962	-35.8725
17	N	2.421352	121.1105	175.0378	41	H	1.079521	120.2741	172.7819
18	C	1.456833	158.0994	-176.175	42	H	1.080307	120.2664	179.5631
19	C	1.409853	120.6383	0.254267	43	H	1.018147	83.20498	-3.516
20	C	1.41239	121.4828	1.231602	44	H	1.090572	110.2736	169.9994
21	C	1.524454	119.7438	179.5326	45	H	1.090448	110.9419	-69.8329
22	F	1.331479	111.1083	0.303277	46	H	1.089461	111.2208	49.97221
23	F	1.331881	109.8913	119.7863	47	H	1.011099	116.6236	-176.547
24	F	1.334154	110.4201	-119.358	48	H	1.008778	117.0865	3.093155

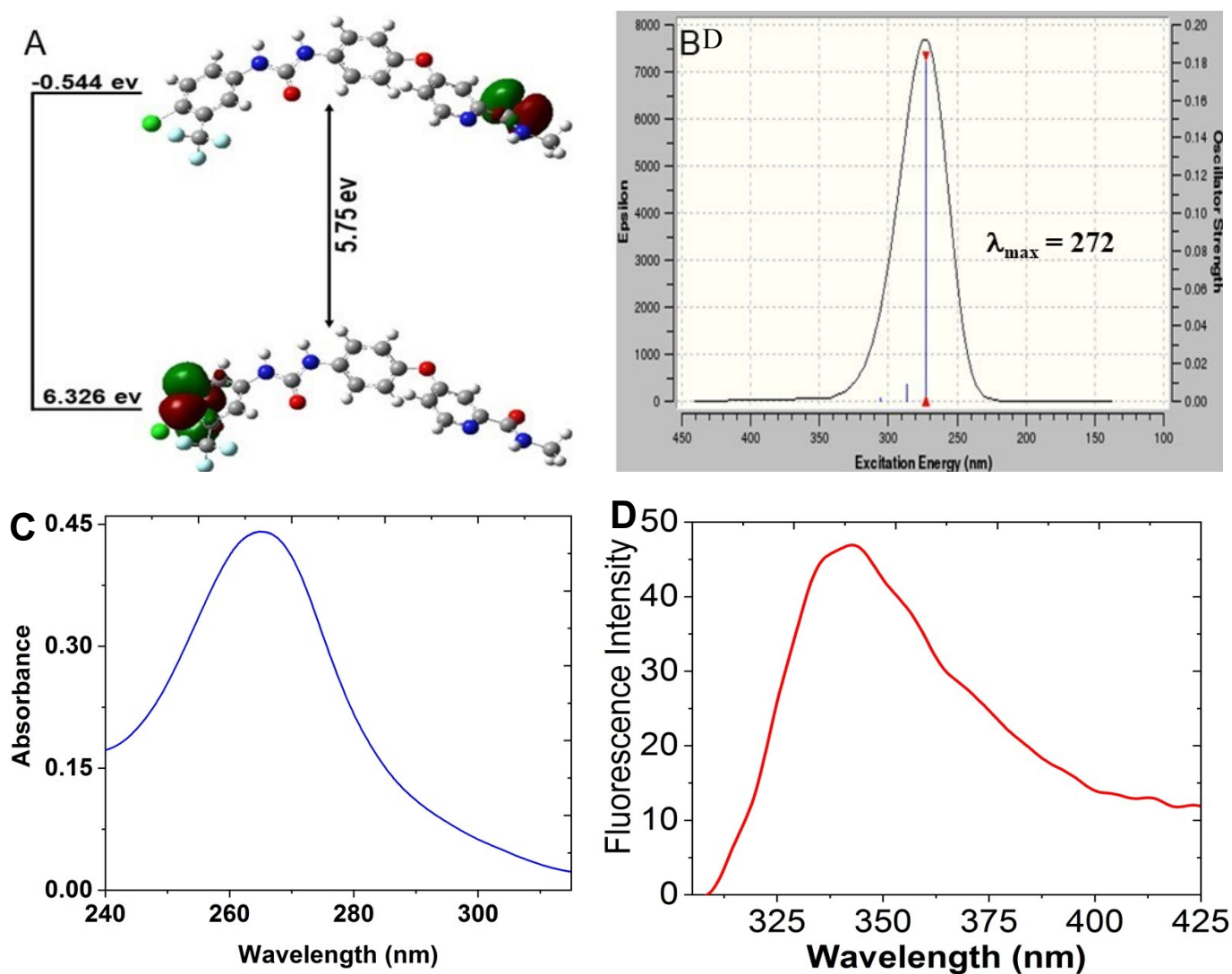


Figure SF1. (A) Excited state HOMO-LUMO energy gap, (B) Theoretically calculated UV-Vis spectra of BAY43-9006 obtained from TDDFT studies, (C) UV-Vis absorption spectrum and (D) fluorescence emission spectrum of BAY43-9006 obtained in ethanolic solution.

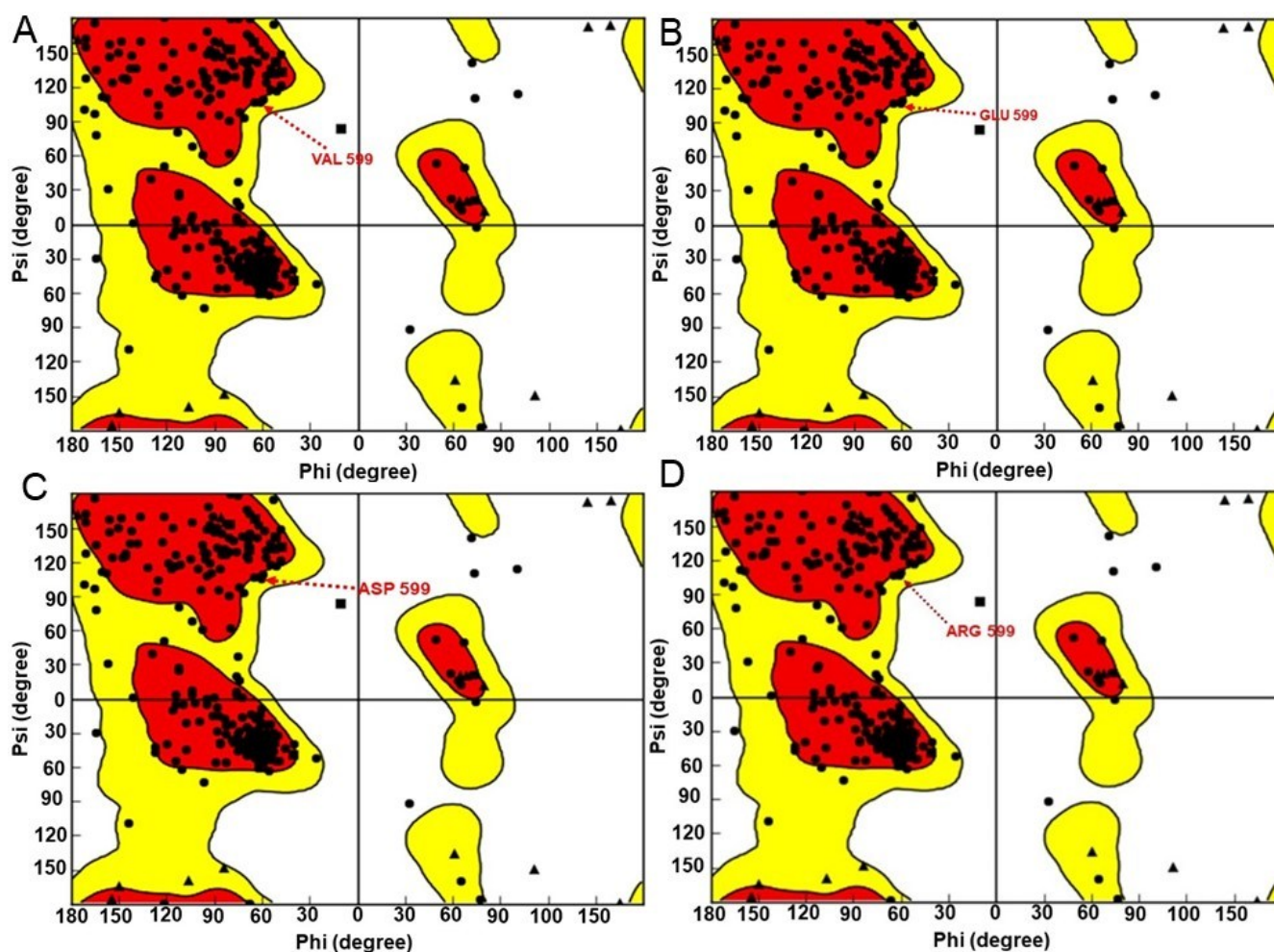
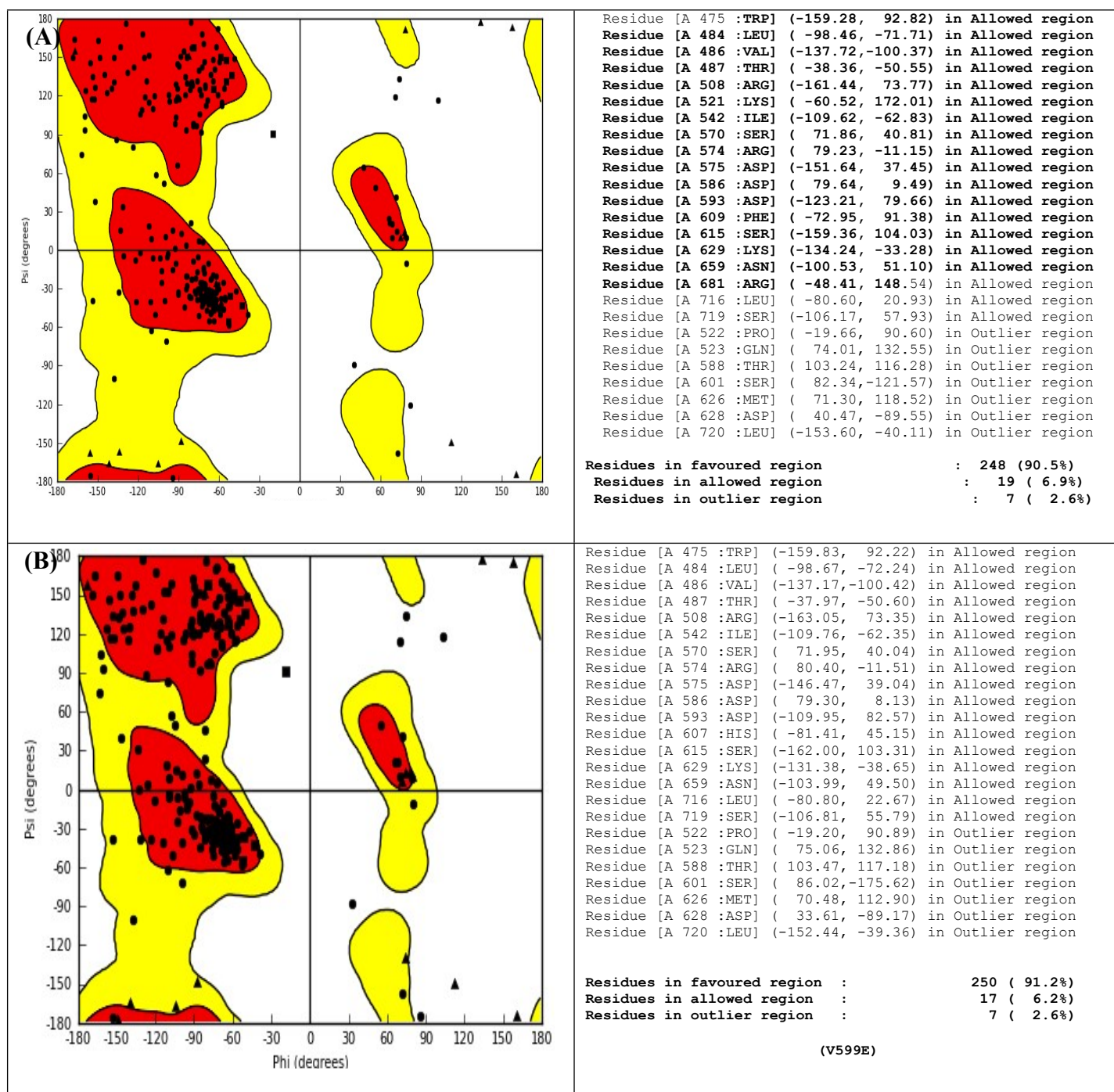
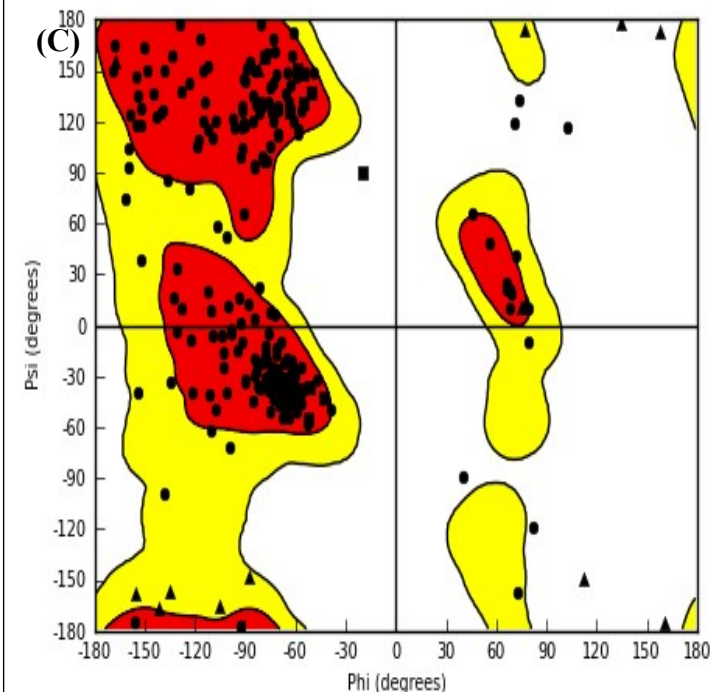


Figure SF 2. (A) Ramachandran plot showing the exact position of 599th residue, (A) for wild type, (B) for V599E, (C) for V599D and (D) for V599R variant of *BRAF* protein.

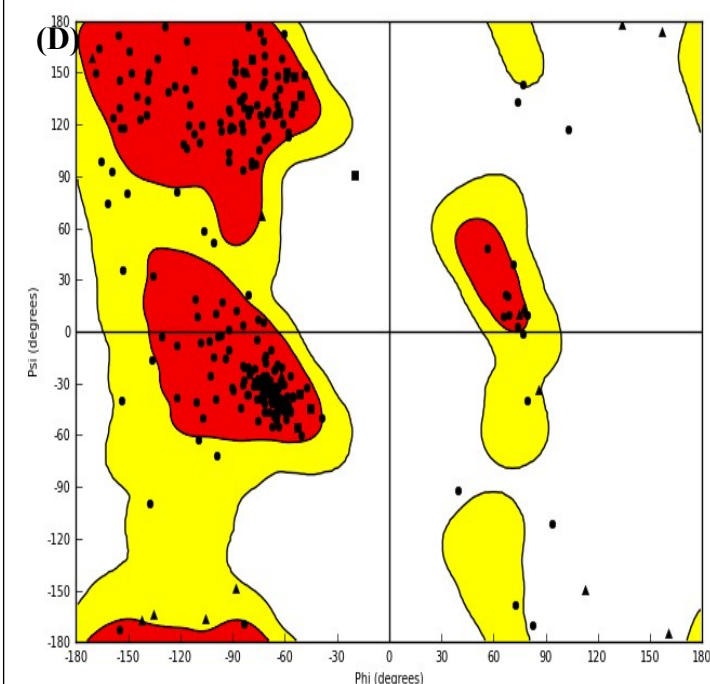
Table ST 2. Ramachandran plot and coordinates of allowed region, **(A)**for wild type, **(B)** for V599E, **(C)**V599Dand **(D)** forV599R showing significant differences in the position of particular amino acid in case of all the four variant of *BRAF* protein.





Residue [A 475 :TRP] (-159.24, 92.85) in Allowed region
 Residue [A 484 :LEU] (-98.39, -71.91) in Allowed region
 Residue [A 486 :VAL] (-137.75, -100.30) in Allowed region
 Residue [A 487 :THR] (-38.41, -50.55) in Allowed region
 Residue [A 508 :ARG] (-161.38, 73.76) in Allowed region
 Residue [A 521 :LYS] (-60.51, 171.97) in Allowed region
 Residue [A 542 :ILE] (-109.67, -62.89) in Allowed region
 Residue [A 570 :SER] (71.85, 40.83) in Allowed region
 Residue [A 574 :ARG] (79.30, -11.14) in Allowed region
 Residue [A 575 :ASP] (-151.58, 37.56) in Allowed region
 Residue [A 586 :ASP] (79.60, 9.49) in Allowed region
 Residue [A 593 :ASP] (-123.03, 79.79) in Allowed region
 Residue [A 607 :HIS] (46.20, 64.80) in Allowed region
 Residue [A 615 :SER] (-159.33, 104.09) in Allowed region
 Residue [A 629 :LYS] (-134.09, -33.62) in Allowed region
 Residue [A 659 :ASN] (-100.65, 51.05) in Allowed region
 Residue [A 681 :ARG] (-48.37, 148.61) in Allowed region
 Residue [A 716 :LEU] (-80.60, 21.16) in Allowed region
 Residue [A 719 :SER] (-106.18, 57.97) in Allowed region
 Residue [A 522 :PRO] (-19.48, 90.47) in Outlier region
 Residue [A 523 :GLN] (74.00, 132.64) in Outlier region
 Residue [A 588 :THR] (103.30, 116.24) in Outlier region
 Residue [A 601 :SER] (82.07, -119.36) in Outlier region
 Residue [A 626 :MET] (71.25, 118.00) in Outlier region
 Residue [A 628 :ASP] (40.41, -89.62) in Outlier region
 Residue [A 720 :LEU] (-153.64, -40.08) in Outlier region

Residues in favoured region : 248 (90.5%)
Residues in allowed region : 19 (6.9%)
Residues in outlier region : 7 (2.6%)



Residue [A 475 :TRP] (-159.29, 92.75) in Allowed region
 Residue [A 484 :LEU] (-98.50, -71.81) in Allowed region
 Residue [A 486 :VAL] (-137.59, -100.19) in Allowed region
 Residue [A 487 :THR] (-38.45, -50.53) in Allowed region
 Residue [A 508 :ARG] (-161.33, 73.73) in Allowed region
 Residue [A 521 :LYS] (-60.49, 171.86) in Allowed region
 Residue [A 542 :ILE] (-109.61, -62.86) in Allowed region
 Residue [A 570 :SER] (71.68, 38.82) in Allowed region
 Residue [A 574 :ARG] (77.13, -1.46) in Allowed region
 Residue [A 575 :ASP] (-152.89, 35.37) in Allowed region
 Residue [A 586 :ASP] (79.49, 9.48) in Allowed region
 Residue [A 593 :ASP] (-121.76, 80.36) in Allowed region
 Residue [A 610 :GLU] (79.75, -40.70) in Allowed region
 Residue [A 614 :GLY] (-73.21, 67.45) in Allowed region
 Residue [A 615 :SER] (-165.12, 98.35) in Allowed region
 Residue [A 626 :MET] (77.09, 142.94) in Allowed region
 Residue [A 629 :LYS] (-136.29, -16.57) in Allowed region
 Residue [A 659 :ASN] (-100.64, 50.83) in Allowed region
 Residue [A 681 :ARG] (-48.33, 148.54) in Allowed region
 Residue [A 716 :LEU] (-80.61, 20.84) in Allowed region
 Residue [A 719 :SER] (-106.21, 57.90) in Allowed region
 Residue [A 522 :PRO] (-19.80, 90.75) in Outlier region
 Residue [A 523 :GLN] (74.16, 132.57) in Outlier region
 Residue [A 588 :THR] (103.38, 116.29) in Outlier region
 Residue [A 601 :SER] (82.87, -170.63) in Outlier region
 Residue [A 608 :GLN] (94.30, -111.90) in Outlier region
 Residue [A 628 :ASP] (39.98, -92.26) in Outlier region
 Residue [A 720 :LEU] (-153.60, -40.11) in Outlier region

Residues in favoured region : 246 (89.8%)
Residues in allowed region : 21 (7.7%)
Residues in outlier region : 7 (2.6%)

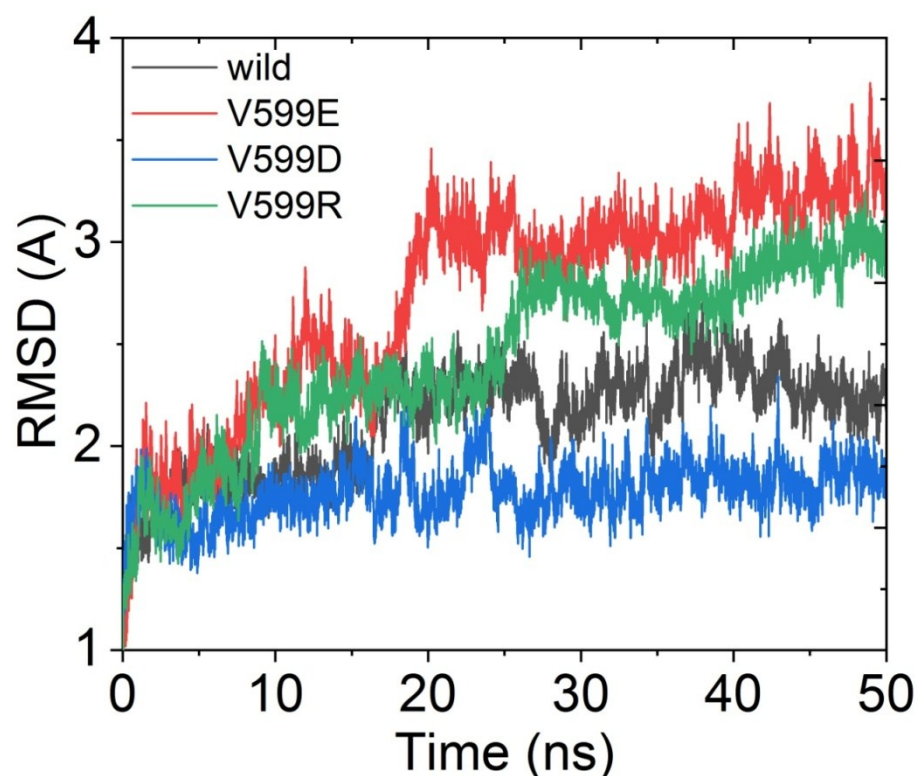


Figure SF3. RMSD plot overlaps of BAY43-9006 bounded *BRAF* variants as obtained during 50 ns Of MD-Simulation studies.

Table ST 3. Maximum RMSF and their corresponding amino acid residues as obtained by MD simulation studies.

S. No	<i>BRAF</i> variant	Residue Location	Maximum RMSF Range (Å)	Involved Residue
1	Wild Type	Loop Region	4.0	Phe-589
		β strand	3.5	Val-589
		Loop Region	3.4	Gly-614
2	V599E	Loop Region	5.2	His-607
		Loop Region	4.7	Gly-414
		Loop Region	3.9	Ala-620
		Loop Region	4.6	Gly-671
3	V599D	β strand	3.2	Gly-477
		Loop Region	3.3	Glu-610
		Loop Region	3.4	Ser-682
4	V599R	Loop Region	3.5	Glu-544
		Loop Region	4.5	Phe-547
		Loop Region	5.2	Ser-604

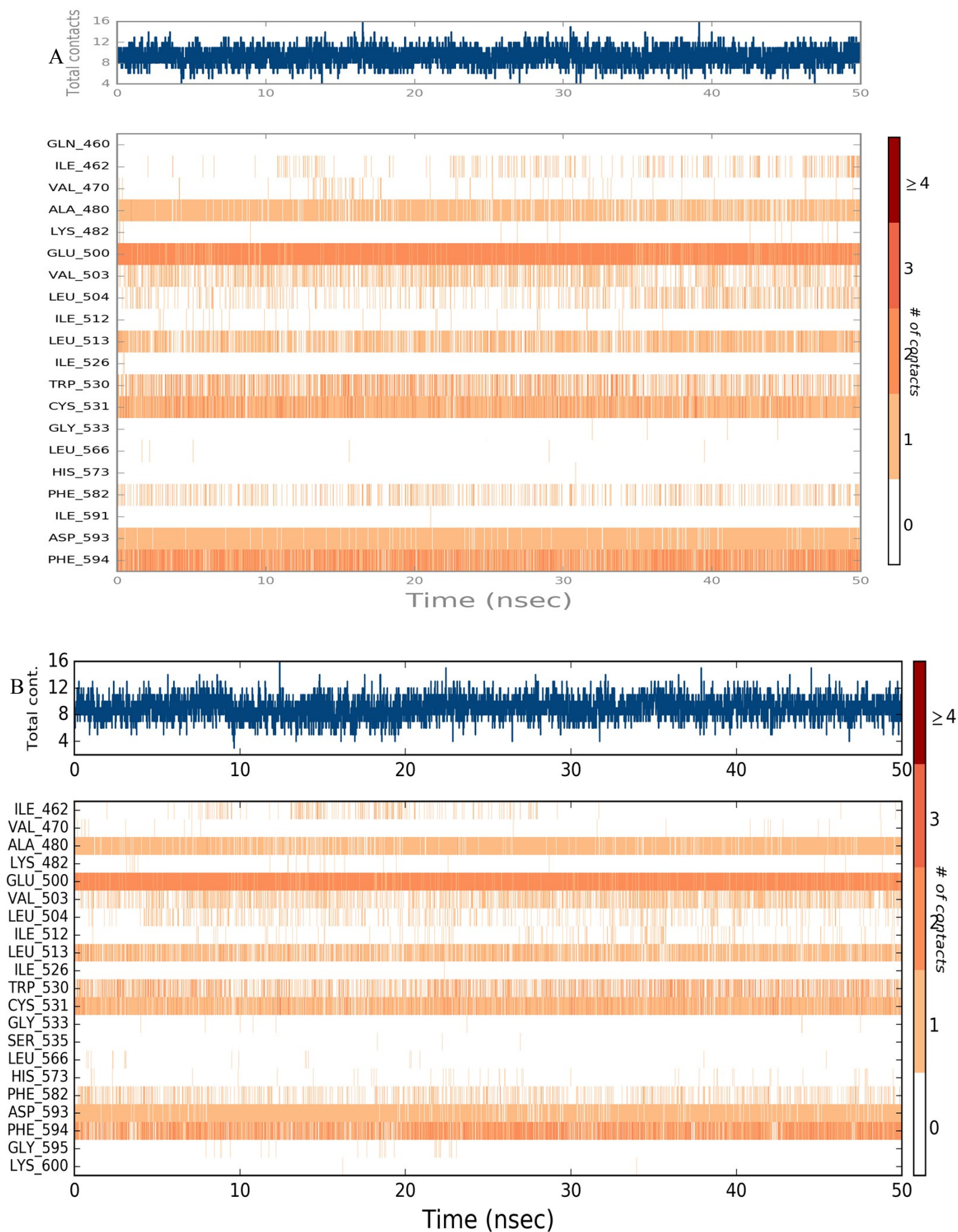


Figure SF4. Protein-Ligand Contacts time line **(A)** for wild type, **(B)** for V599E, **(C)**V599Dand **(D)** forV599R showing differences among all the four variants of *BRAF* proteins

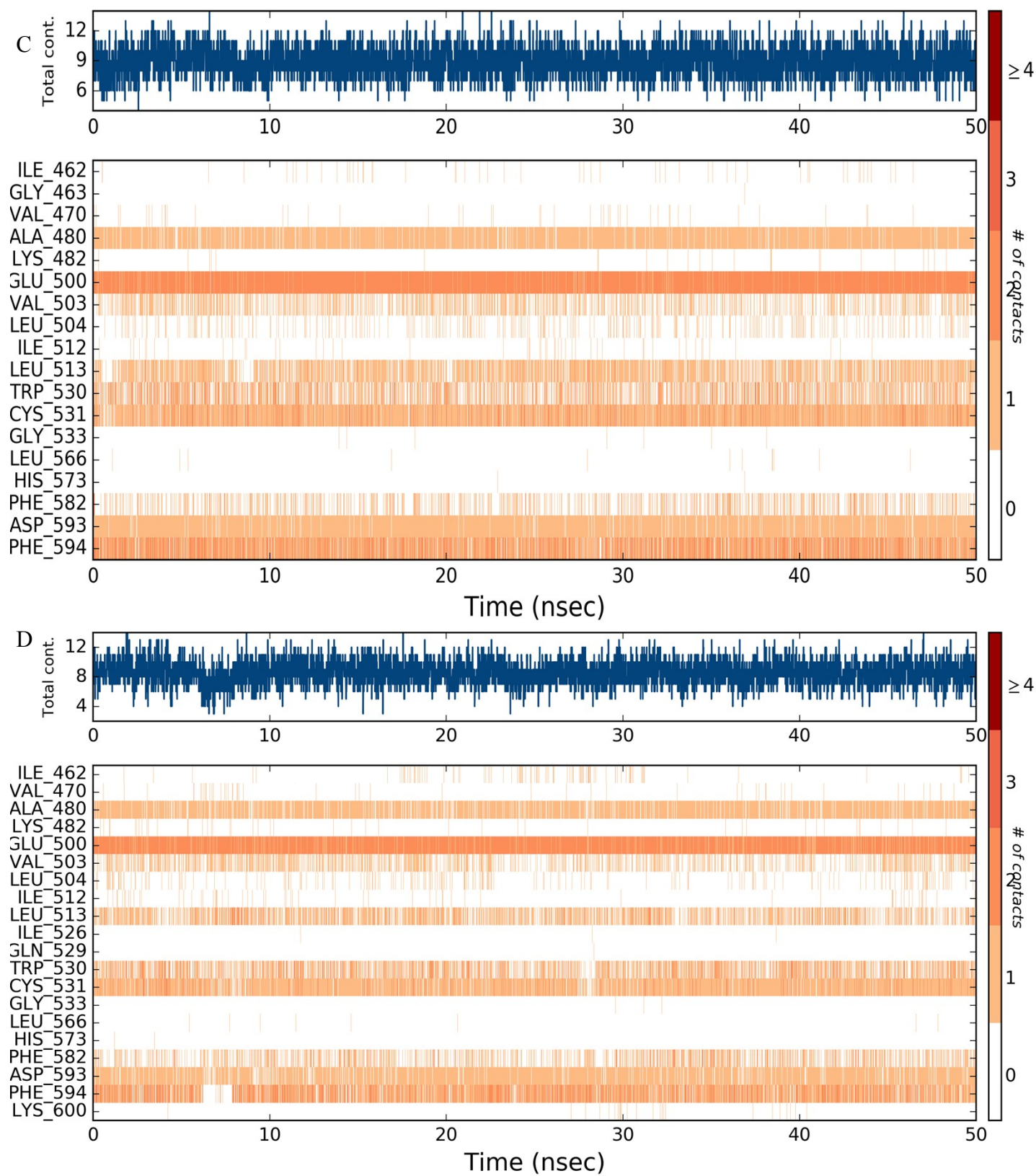


Figure SF 4. (Continuous)

Table ST 4. Global parameters of RIN analysis for all the four variants of BAY43-9006 bounded *BRAF* protein

S. no	<i>BRAF</i> Variant	No of Eges	Diameter	Radius	Average Degree	Average Shortest Path
1	Wild	1041	13	7	7.54	5.69
2	V599E	1041	13	7	7.54	5.68
3	V599D	1043	13	7	7.56	5.67
4	V599R	1051	13	7	7.62	5.65

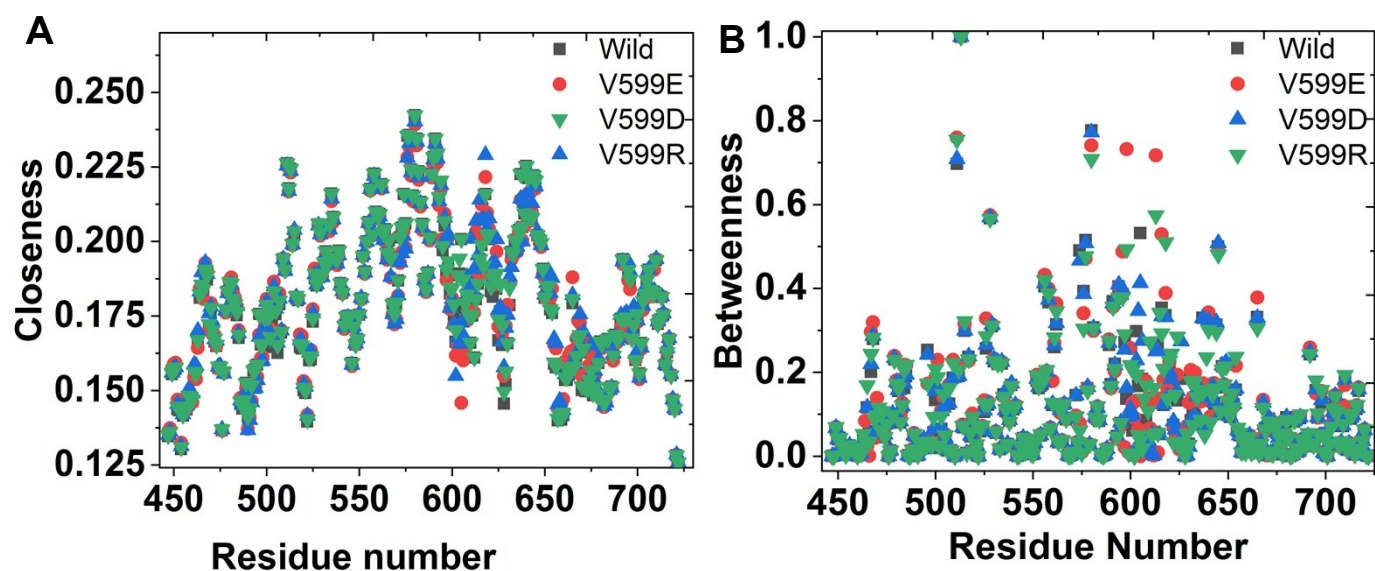


Figure SF5. Closeness centrality (A) and betweenness centrality (B) plots overlaps for Protein- BAY43-9006 complexes of wild, V599E, V599D and V599R variants of *BRAF* proteins