Supporting Information: Ensemble-based replica exchange alchemical free energy methods: the effect of protein mutations on inhibitor binding

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The main article describes the application of TIES, TIES-λ-REST2, and TIES-λ-REST2-M to predict binding free energy changes of inhibitors cause by protein mutations. We used different T_{eff} values, the highest effective REST2 temperature, for the receptor and the complexes to check the accuracy and convergence of the free energy calculations. The Supporting Information provides, in order of appearance, more details of the calculated free energies from the V555M (Table S1), I538V (Table S2), and N540S (Table S3) mutations with the highest T_{eff} of 600 K for the receptor and 600 K/1500 K for the complexes; as well as detailed results for V555M (Table S4), I538V (Table S5), and N540S (Table S6) mutations with the highest T_{eff} of 1500 K for the receptor and 3000 K for the complexes. Table S7 shows the occupancies of hydrogen bonds between the inhibitors and the protein.

Table S1: Free energy predictions for the WT-V555M transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-linear				0.11(0.27)	-0.42(0.06)	-0.46(0.05)	-3.56(0.31)	-2.76(0.12)	-2.70(0.12)	-1.75(0.33)
AZD4547-bent				-4.00(0.38)	-1.12(0.05)	-1.18(0.04)	0.55(0.41)	-2.07(0.11)	-1.98(0.12)	
BGJ-398	-3.45(0.16)	-3.18(0.10)	-3.16(0.11)	-0.43(0.41)	0.48(0.07)	0.44(0.06)	-3.02(0.44)	-3.66(0.12)	-3.60(0.12)	-1.19(0.08)
TKI258				-3.71(0.19)	-2.01(0.08)	-2.05(0.07)	0.26(0.25)	-1.17(0.13)	-1.11(0.13)	0.97(0.22)
JNJ42756493				1.74(0.34)	0.81(0.12)	0.76(0.10)	-5.19(0.38)	-3.99(0.16)	-3.92(0.15)	-3.08(0.17)

Table S2: Free energy predictions for the WT-I538V transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-linear				-0.99(0.23)	-0.52(0.05)	-0.56(0.04)	0.25(0.33)	0.09(0.11)	0.05(0.11)	-2.11(0.32)
BGJ-398	-0.74(0.24)	-0.43(0.10)	-0.51(0.10)	-1.18(0.26)	-0.89(0.05)	-0.96(0.04)	0.44(0.35)	0.46(0.11)	0.45(0.11)	-0.74(0.21)
TKI258				-0.09(0.30)	-0.90(0.08)	-0.89(0.07)	-0.65(0.38)	0.47(0.13)	0.38(0.12)	-1.91(0.13)
JNJ42756493				-1.36(0.24)	-0.73(0.07)	-0.79(0.06)	0.62(0.34)	0.30(0.12)	0.28(0.12)	-2.18(0.10)

Table S3: Free energy predictions for the WT-N540S transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-linear	2.98(0.36)	2.63(0.12)	2.62(0.12)	3.41(0.23)	1.72(0.07)	1.67(0.07)	-0.43(0.43)	0.91(0.14)	0.95(0.14)	-0.76(0.33)
BGJ-398				3.98(0.38)	1.50(0.07)	1.46(0.06)	-1.00(0.52)	1.13(0.14)	1.16(0.13)	0.25(0.19)
TKI258				4.75(0.48)	1.61(0.08)	1.51(0.08)	-1.77(0.60)	1.02(0.14)	1.11(0.14)	-0.9(0.15)
JNJ42756493				3.85(0.27)	1.57(0.08)	1.51(0.08)	-0.87(0.45)	1.06(0.14)	1.11(0.14)	-1.75(0.21)

Table S4: Free energy predictions for the WT-V555M transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time but the highest T_{eff} increased to 1500 K/3000 K**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-linear	-3.45(0.16)	-1.65(0.06)	-1.68(0.05)	0.11(0.27)	0.20(0.04)	0.14(0.04)	-3.56(0.31)	-1.85(0.07)	-1.82(0.06)	-1.75(0.33)
AZD4547-bent				-4.00(0.38)	-0.58(0.04)	-0.57(0.04)	0.55(0.41)	-1.07(0.07)	-1.11(0.06)	
BGJ-398				-0.43(0.41)	0.27(0.05)	0.28(0.04)	-3.02(0.44)	-1.92(0.08)	-1.96(0.06)	-1.19(0.08)
TKI258				-3.71(0.19)	-0.24(0.05)	-0.26(0.04)	0.26(0.25)	-1.41(0.08)	-1.42(0.06)	0.97(0.22)
JNJ42756493				1.74(0.34)	1.23(0.10)	1.19(0.10)	-5.19(0.38)	-2.88(0.12)	-2.87(0.11)	-3.08(0.17)

Table S5: Free energy predictions for the WT-I538V transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time but the highest T_{eff} increased to 1500 K/3000 K**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-linear	-0.74(0.24)	-0.90(0.06)	-0.87(0.06)	-0.99(0.23)	-0.78(0.06)	-0.83(0.04)	0.25(0.33)	-0.12(0.08)	-0.04(0.07)	-2.11(0.32)
BGJ-398				-1.18(0.26)	-0.91(0.05)	-0.96(0.04)	0.44(0.35)	0.01(0.08)	0.09(0.07)	-0.74(0.21)
TKI258				-0.09(0.30)	-0.91(0.06)	-0.99(0.06)	-0.65(0.38)	0.01(0.08)	0.12(0.08)	-1.91(0.13)
JNJ42756493				-1.36(0.24)	-0.89(0.04)	-0.98(0.03)	0.62(0.34)	-0.01(0.07)	0.11(0.07)	-2.18(0.10)

Table S6: Free energy predictions for the WT-N540S transformation using TIES and schemes λR2 and λR2-M (**up to 4 ns simulation time but the highest T_{eff} increased to 1500 K/3000 K**). Corresponding experimental values are also shown. The values in brackets denote the error bars. All values are in kcal/mol.

Drug	ΔG_{alch}						$\Delta\Delta G_{alch}$			$\Delta\Delta G_{exp}$
	rec			com						
	TIES	λR2	λR2-M	TIES	λR2	λR2-M	TIES	λR2	λR2-M	
AZD4547-normal	2.98(0.36)	1.61(0.10)	1.56(0.10)	3.41(0.23)	0.89(0.05)	0.82(0.05)	-0.43(0.43)	0.72(0.11)	0.74(0.11)	-0.76(0.33)
BGJ-398				3.98(0.38)	0.94(0.05)	0.89(0.04)	-1.00(0.52)	0.67(0.11)	0.67(0.11)	0.25(0.19)
TKI258				4.75(0.48)	0.90(0.06)	0.84(0.06)	-1.77(0.60)	0.71(0.12)	0.72(0.12)	-0.9(0.15)
JNJ42756493				3.85(0.27)	0.89(0.07)	0.84(0.06)	-0.87(0.45)	0.72(0.12)	0.72(0.12)	-1.75(0.21)

Table S7: Occupancies (%) of the hydrogen bonds between glutamic acid and analine residues of the hinge region and the inhibitor for all complexes using the standard TIES as well as λ-REST2 simulations at both end-points of the alchemical transformations studied. "hot" denotes if the residue is included in the REST2 region or not.

Mutant	Drug	ALA:100					GLU:98‡				
		H-bond occupancy (%)†					H-bond occupancy (%)†				
		"hot"	$\lambda = 0$		$\lambda = 1$		"hot"	$\lambda = 0$		$\lambda = 1$	
			TIES	λR2	TIES	λR2		TIES	λR2	TIES	λR2
V2M	AZD-l	n	67.4, 3.6	69.4, 5.7	66.6, 5.8	67.0, 5.3	y	91.4	88.6	87.9	84.0
	AZD-b	n	45.8, 1.5	54.5, 3.2	55.5, 2.4	48.9, 1.9	y	89.9	88.8	84.4	89.3
	BGJ	n	40.4, 14.0	44.3, 24.7	51.1, 13.4	50.2, 10.0	y	-	-	-	-
	TKI	n	69.0	68.6	65.5	59.2, 0.4	y	30.6	20.7	29.2	15.0
	JNJ	n	21.2	25.2	28.4	20.8	y	-	-	-	-
I2V	AZD-l	n	68.8, 4.7	68.5, 4.8	68.4, 6.2	71.3, 6.2	n	90.4	90.3	90.0	89.0
	BGJ	n	39.4, 30.2	39.7, 32.3	50.6, 16.6	41.5, 29.4	n	-	-	-	-
	TKI	y	75.6	69.2, 0.4*, 0.2	57.7	52.9, 6.7*, 2.2*,2.2*, 1.0*, 0.1	n	27.4	30.3	27.8	16.9
	JNJ	n	24.0	25.4	21.3	27.4	n	-	-	-	-
N2S	AZD-l	n	70.5, 6.4	67.4, 6.4	68.9, 7.6	70.0, 7.4	y	90.8	88.0	88.3	89.2
	BGJ	n	39.3, 26.3	41.8, 31.0	41.4, 24.8	34.3, 36.5	y	-	-	-	-
	TKI	y	70.3	50.3, 2.6*, 1.4*, 1.3, 0.4*	57.2	51.8, 4.6*, 3.4*, 1.4*,1.0*, 0.1	y	28.5	15.9	32.7	20.4, 1.0*
	JNJ	n	19.6	25.8	18.3	27.0	y	-	-	-	-

‡ GLU:98 does not form any H-bond with BGJ-298 and JNJ42756493.

† Asterisk denotes flipped conformations of the inhibitor TKI258.