Report

Free energy calculations for inhabitor mutations Me-hydan$\Longrightarrow$N-hydan, hydan$\Longrightarrow$O-hydan, and N-hydan$\Longrightarrow$O-hydan have been carried out in both glycogen phosphorylase and solution. The conclusion is that N-hydan, O-hydan and O-hydan are the favored sides in these three mutations in both the protein and solution environment. Analysis of the free energy components by the unit of residues reveals that ASP283 makes the most important contributions to the free energy difference in both mutations.

I. ANALYSIS OF ENDPOINT STRUCTURES

A. Hydrogen bonds around the ligands

The interactions of the O7 and O8 of the ligand with the environment, i.e. the proteins and waters, are conjectured to play an important role on stablizing the ligand and contributing to the free energy change of a mutation on the N1 group.

1. Around O7: Water 61 and 66

It was observed that water 61 and 66 stay very stably in their positions. During the simulations, the two hydrogens of 61 formed two stable hydrogen bonds with OD2 of ASP^{283} and O7 of the ligand. We noticed that occasionally they rotated to allow their two hydrogen atoms to exchange positions. For example, in the O-hydan case, from time zero to approximately 200 ps, H2 formed a hydrogen bond with OD2, H1 formed the other one with O7. At 200 ps, H1 and H2 exchanged their positions and from then to 500 ps the whole water molecule remained unchanged. For 66, its H2 first interacted with OH2 of 61, at about 80 ps it started to interact with OD1 of ASP^{283}, and this interaction lasted until 440 ps after which we observed that it returned to OH2 of 61.

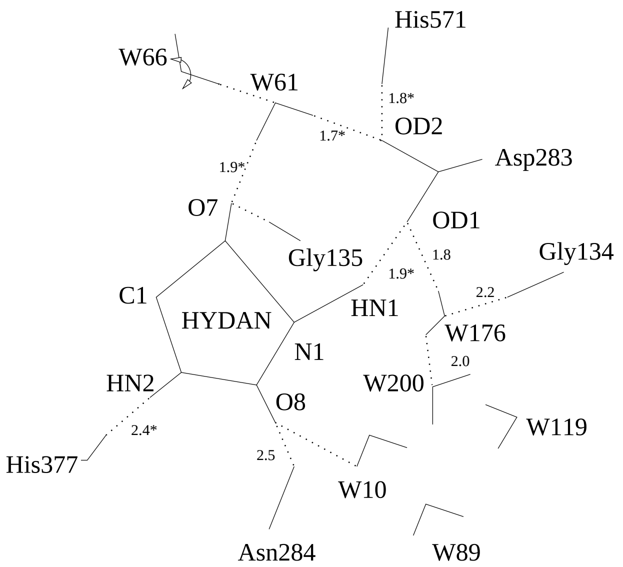

FIG. 1. Formation of hydrogen bonds around hydan.

2. The water world around O8

There are a group of water molecules around O8. Since the void formed between the ligand and protein is relatively more spacious around O8, this place appears to be able to accomodate more waters, though it has been proven that sometimes filling a cavity in protein with waters does not necessarily increase the thermal stability of the protein. (We observed that in the case of hydan, O8 does not seem to form any *stable* hydrogen bonds with water, in contrast to the other three cases where O8 always attracts considerably some waters. This fact, however, does not seem to challenge the fact that hydan is in actuality more favorable than Me-hydan and N-hydan.) And the lack of a charged residue like ASP^{283} in the neighborhood (there is a protonated ASP around) conveniences the motion of waters and seems to make possible a water channel by which water molecules could be observed entering and leaving the void in a long time simulation.

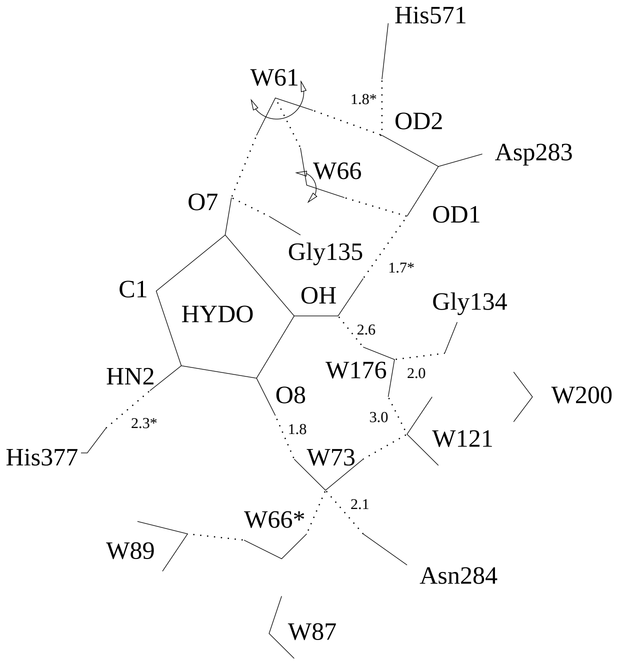

FIG. 2. Formation of hydrogen bonds around O-hydan.

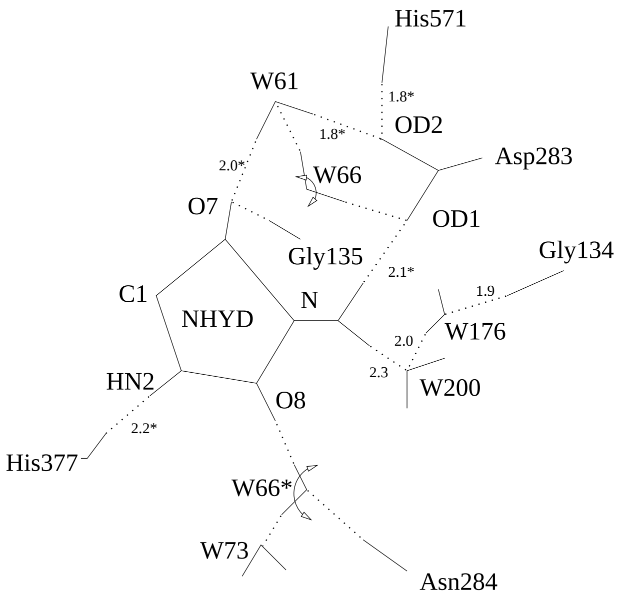

FIG. 3. Formation of hydrogen bonds around N-hydan.

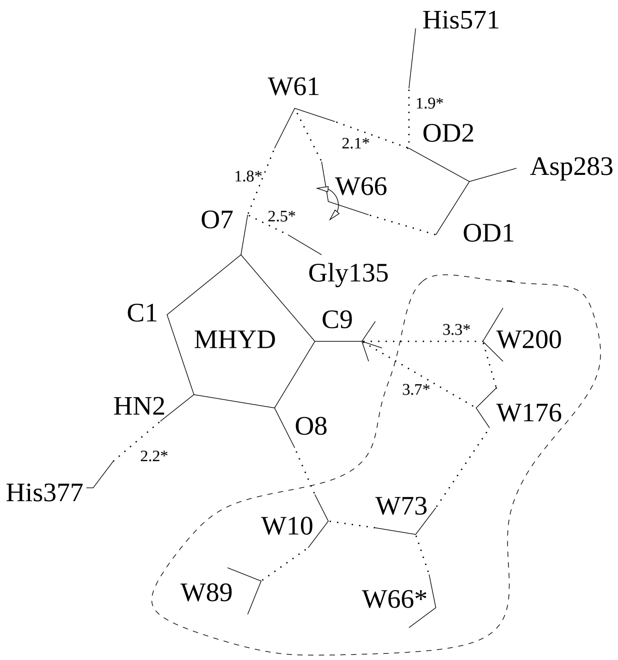

FIG. 4. Formation of hydrogen bonds around Me-hydan.

B. RMS deviations

TABLE I. The RMS deviations (Å) from different structures. Averaged over the heavy atoms only (100ps).

Inhibitor	run	ligand	main chain	side chain	crystal water	solvent water	active(main)	active(side)
Hydan								
crys.	2	0.54	0.51	0.77	2.36	7.72	0.56	0.85
init.	2	0.48	0.47	0.58	1.65	3.80	0.44	0.60
aver.	2	0.33	0.34	0.43	1.05	2.41	0.33	0.44
N-hydan								
crys.	2	0.49	0.54	0.77	3.34	—-	0.63	0.84
init.	2	0.50	0.46	0.56	1.15	1.99	0.46	0.56
aver.	2	0.29	0.33	0.40	0.75	1.25	0.32	0.40
O-hydan								
crys.	2	—-	0.52	0.80	3.26	—-	0.59	0.88
init.	2	0.43	0.47	0.61	1.60	4.04	0.45	0.62
aver.	2	0.34	0.34	0.44	0.99	2.40	0.34	0.44
Me-Hydan								
crys.	2	0.45	0.49	0.74	2.41	—-	0.52	0.85
init.	2	0.41	0.46	0.56	1.19	1.91	0.44	0.55
aver.	2	0.30	0.33	0.40	0.77	1.26	0.32	0.40

TABLE II. The RMS deviations (Å) of loops 281-288 and 378-384 from different reference structures. Averaged over the heavy atoms only (100ps).

Inhibitors	Crys. main	side	End main	side	Aver. main	side
Hydan						
Loop 281	0.58	0.91	0.45	0.60	0.35	0.51
Loop 378	1.18	1.54	0.44	0.64	0.37	0.50
N-hydan						
Loop 281	0.88	1.29	0.50	0.61	0.33	0.45
Loop 378	1.06	1.44	0.67	0.85	0.45	0.65
O-hydan						
Loop 281	0.90	1.40	0.49	0.70	0.38	0.54
Loop 378	0.87	1.30	0.61	1.11	0.45	0.71
Me-hydan						
Loop 281	0.58	0.87	0.46	0.58	0.36	0.41
Loop 378	0.85	1.38	0.55	0.75	0.37	0.54

TABLE III. The RMS deviations (Å) of waters near the ligand from their final positions, representing the range of their motion. Averaged over the heavy atoms only (100ps).

waters	Hydan	N-hydan	O-hydan	Me-Hydan
010 WATA	2.01	0.97	1.20	2.71
061 WATA	0.45	0.50	0.54	0.85
066 WATA	0.80	0.86	1.44	1.05
076 WATA	2.77	0.66	0.60	0.89
108 WATA	0.67	0.55	0.65	0.74
111 WATA	0.77	1.90	0.64	0.76
112 WATA	0.64	0.79	0.58	0.65
138 WATA	1.98	0.61	2.50	2.14
139 WATA	0.69	0.42	0.46	1.30
176 WATA	0.84	0.53	0.86	0.54
200 WATA	0.54	0.59	2.69	0.52
066 WATE	1.14	0.79	2.08	3.53
089 WATE	1.01	0.84	2.44	2.44
119 WATE	0.93	2.94	2.18	1.06

C. Interaction energies by residues

There are some residues around the ligand which have acidic and basic side chains: Asp283, Glu672, Lys574. The OD1 and OD2 of Asp, as well as the OE1 and OE2 of Glu, have a negative charge of -0.76, whereas the three HZ* atoms of Lys carry a positive charge of 0.33.

We calculated the interaction energies of the five-member ring of the ligands with the surrounding residues and waters. The results are listed in Table V. We noticed that the electrostatic interaction energy between O-hydan and Asp283 is very different from those between N-hydan or Me-Hydan and Asp283. This may imply that the free energy change(which incorporates the entropic effect) due to a mutation between the O-hydan and N-hydan (or Me-Hydan, and probably hydan0, I have to do an independent simulation about hydan and hydan0 in order to complete Table V) could be very different from that due to a mutation between any pair between the non-O-hydan ligands, e.g., Me-hydan→N-hydan. Of course this conjecture may not be always true since the free energy components are usually path-dependent.

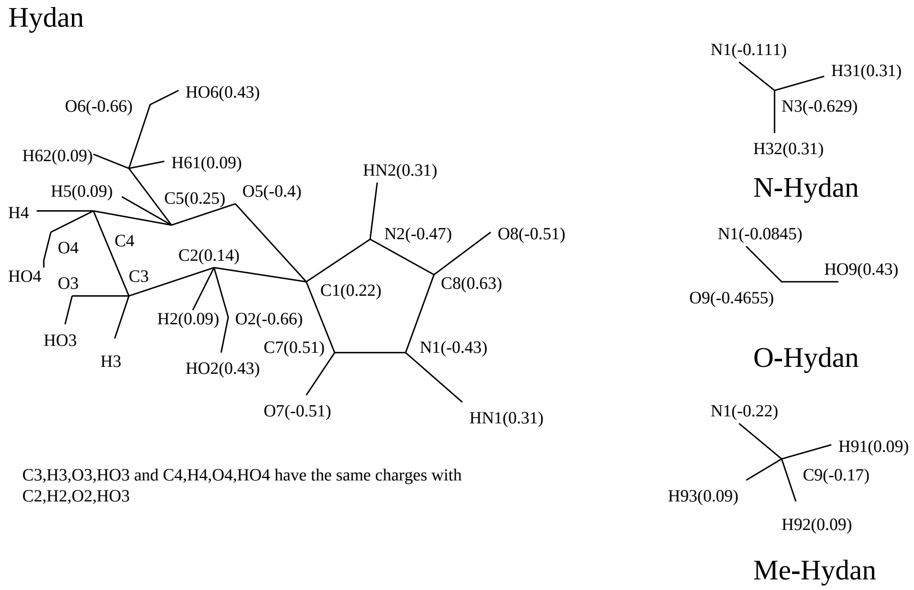

FIG. 5. Selected atomic charges of the four inhibitors.

The two residues, Lys574 and Glu672, are relatively further away from the ligand site. However, their electrostatic interactions with the ligand contributes significantly to the total interaction energy, thus make it a necessity to take them into account while analyzing the results. Even if the contributions of the two somehow cancel each other, their electrostatic effect on the ligand could likely be important since they are not very far away from the ligand.

TABLE IV. Some atomic charges of the ligands and protein residues. From this table, we cannot see any dramatical difference between the charges of O-hydan and those of other inhibitors. Therefore, why the O-hydan interaction energy with ASP283 is extraodinarily negative remains yet a question to answer.

Atoms	C7	O7	C8	O8	N2	HN2	N1	HN1	N3	HN31,2	O9	HO9	C9	H91,2,3
H-hydan	0.51	-0.51	0.63	-0.51	-0.47	0.31	-0.43	0.31	—	—	—	—	—	—
N-hydan	0.51	-0.51	0.63	-0.51	-0.47	0.31	-0.111	—	-0.629	0.31	—	—	—	—
O-hydan	0.51	-0.51	0.63	-0.51	-0.47	0.31	-0.0845	—	—	—	-0.4655	0.43	—	—
Me-Hydan	0.51	-0.51	0.63	-0.51	-0.47	0.31	-0.22	—	—	—	—	—	-0.17	0.09

TABLE V. Van der Waals and electrostatic interaction energies (kcal/mol) between important residues and the five-member ring of Hydan, O-hydan, Me-hydan and N-hydan. In the parentheses are the standard deviations.

	Hydan		O-hydan		Me-hydan		N-hydan	
Residue	vw	elec	vw	elec	vw	elec	vw	elec
W61	0.65(0.94)	-4.01(1.48)	0.01(0.72)	-1.49(2.34)	0.91(1.04)	-6.15(1.65)	0.47(0.90)	-4.57(2.16)
W66	-0.12(0.08)	-1.21(0.40)	-0.33(0.13)	0.16(0.87)	-0.10(0.07)	-1.18(0.35)	-0.17(0.13)	-1.03(0.53)
W176	-0.42(0.27)	1.79(0.79)	-0.27(0.23)	-0.88(0.86)	-0.27(0.24)	0.97(0.31)	-0.32(0.22)	0.96(0.44)
W200	-0.27(0.08)	0.36(0.54)	-0.27(0.13)	2.05(0.78)	0.05(0.45)	-0.22(0.20)	0.12(0.54)	-1.24(1.04)
W10	0.12(0.76)	-1.87(2.59)	-0.05(0.07)	-0.78(0.61)	-0.21(0.07)	1.99(0.73)	-0.02(0.01)	-0.16(0.12)
Asp283	0.85(3.02)	$\underline{0.06}$(1.35)	0.91(1.67)	$\underline{-6.36}$(2.77)	-1.56(0.53)	$\underline{13.25}$(1.54)	-0.98(1.00)	$\underline{8.85}$(2.10)
Asn284	-2.36(0.45)	-3.40(1.13)	-3.01(0.43)	-3.53(1.20)	-3.03(0.36)	-1.40(0.67)	-3.06(0.36)	-2.14(0.77)
Hse377	-1.10(0.38)	-1.62(1.16)	-1.13(0.49)	-2.10(1.22)	-0.68(0.88)	-2.73(1.36)	-0.92(0.60)	-2.39(1.19)
Thr378	-1.29(0.19)	-0.53(0.43)	-1.30(0.21)	-0.23(0.49)	-1.40(0.34)	-0.92(0.39)	-1.36(0.18)	-0.60(0.37)
Lys574	-0.22(0.05)	-10.87(0.97)	-0.18(0.03)	-7.79(0.87)	-0.23(0.04)	-11.0(0.97)	-0.18(0.03)	-11.0(0.79)
Glu672	-0.19(0.03)	8.02(0.64)	-0.16(0.02)	5.92(0.59)	-0.17(0.02)	7.75(0.62)	-0.18(0.02)	8.35(0.58)
Gly135	-0.29(0.76)	-4.06(0.79)	-0.78(0.56)	-3.05(0.52)	-0.60(0.69)	-4.08(0.66)	-0.68(0.66)	-3.93(0.57)
Leu136	-2.07(0.36)	0.03(0.33)	-2.81(0.45)	-1.43(0.56)	-2.63(0.36)	-0.31(0.51)	-2.98(0.42)	-0.73(0.53)

TABLE VI. Van der Waals and electrostatic interaction energies (kcal/mol) between important residues and the ligands: Hydan, O-hydan, Me-hydan and N-hydan. In the parentheses are the standard deviations.

	Hydan		O-hydan		Me-hydan		N-hydan	
Residue	vw	elec	vw	elec	vw	elec	vw	elec
Wat10	0.09(0.75)	-1.73(2.50)	-0.06(0.07)	-0.59(0.57)	-0.22(0.08)	1.67(0.69)	-0.02(0.01)	-0.13(0.08)
Wat61	0.28(0.93)	-3.83(1.30)	-0.27(0.73)	-2.58(1.31)	0.51(1.02)	-6.03(1.43)	0.06(0.88)	-4.66(1.43)
Wat66	-0.17(0.09)	-0.91(0.35)	-0.43(0.13)	0.15(0.73)	-0.15(0.08)	-0.45(0.32)	-0.22(0.14)	-0.87(0.42)
Wat108	-0.01(0.78)	-3.96(2.27)	-0.04(0.76)	-4.50(2.26)	0.50(1.09)	-6.49(2.61)	1.30(1.22)	-7.76(1.76)
Wat139	0.39(0.99)	-5.72(1.40)	0.82(1.16)	-4.67(1.70)	1.82(1.44)	-10.55(1.7)	0.45(1.10)	-5.55(1.47)
Wat176	-0.52(0.26)	1.24(0.79)	-0.31(0.23)	-1.28(0.82)	-0.30(0.23)	0.28(0.29)	-0.36(0.22)	0.56(0.40)
Wat200	-0.29(0.08)	0.01(0.55)	-0.29(0.13)	1.73(0.72)	0.03(0.45)	-0.75(0.24)	0.09(0.54)	-1.65(1.04)
Wat89*	-0.10(0.08)	0.43(0.56)	-0.06(0.03)	0.38(0.46)	0.32(0.97)	-4.35(3.28)	-0.07(0.02)	-0.18(0.27)
Asp283	-0.25(1.34)	-5.68(3.11)	0.58(1.66)	-12.61(2.7)	-1.81(0.53)	3.72(1.47)	-1.25(1.00)	2.52(2.15)
Asn284	-2.83(0.77)	-6.48(1.77)	-3.33(0.79)	-6.95(1.69)	-3.32(0.72)	-4.52(1.43)	-3.43(0.68)	-5.81(1.36)
Hse377	-1.88(1.26)	-12.59(1.8)	-2.42(1.36)	-11.94(2.5)	-1.44(1.58)	-12.73(2.1)	-1.16(1.44)	-14.34(1.8)
Thr378	-1.96(0.26)	-0.20(0.56)	-2.04(0.26)	0.25(0.63)	-2.16(0.35)	-0.36(0.62)	-2.13(0.25)	-0.05(0.55)
Lys574	-0.70(0.13)	-1.76(2.19)	-0.64(0.11)	1.38(1.87)	-0.78(0.14)	2.64(2.01)	-0.60(0.09)	-3.45(1.49)
Glu672	0.66(1.74)	-17.99(7.4)	-0.20(1.28)	-16.76(5.4)	0.50(1.42)	-18.19(4.84)	-0.29(1.24)	-10.46(3.9)
Gly135	-1.53(0.82)	-4.07(1.01)	-1.93(0.64)	-3.14(0.91)	-1.81(0.77)	-2.26(0.96)	-1.96(0.68)	-3.99(0.85)
Leu136	-3.68(0.51)	-0.80(0.35)	-4.50(0.52)	-2.18(0.58)	-4.08(0.52)	-0.84(0.47)	-4.92(0.47)	-1.34(0.52)
sum	-20.50	-75.70(-77.76)	-23.57	-72.36(-74.87)	-20.39	-70.47(-76.52)	-23.24	-75.00(-77.40)
solution	-12.96	-70.60	-13.49	-78.00	-15.95	-66.00	-15.12	-69.99
Wat10	0.42(0.93)	-3.88(1.83)	-0.01(0.01)	-0.05(0.14)	0.92(1.17)	-6.26(2.42)	-0.02(0.01)	-0.12(0.10)
Wat61	0.40(0.96)	-3.94(1.40)	-0.40(0.66)	-2.30(1.32)	0.64(1.16)	-5.90(1.75)	0.02(0.90)	-4.56(1.38)
Wat66	-0.16(0.23)	-0.97(0.60)	-0.38(0.15)	0.11(0.75)	-0.21(0.12)	-0.54(0.39)	-0.30(0.13)	-0.77(0.46)
Wat108	0.32(1.00)	-4.71(2.58)	0.77(1.12)	-6.77(2.32)	0.75(1.18)	-7.44(2.26)	1.03(1.25)	-6.91(2.23)
Wat139	0.17(0.97)	-5.37(1.61)	0.68(1.11)	-4.79(1.94)	1.74(1.44)	-10.70(1.8)	0.41(1.05)	-5.07(1.68)
Wat176	-0.50(0.23)	0.78(0.89)	-0.22(0.40)	-1.38(0.78)	-0.30(0.23)	0.22(0.30)	-0.38(0.17)	0.48(0.37)
Wat200	-0.29(0.08)	0.18(0.44)	-0.13(0.15)	0.68(1.16)	0.03(0.44)	-0.75(0.24)	0.07(0.56)	-1.72(1.07)
Wat89*	-0.22(0.06)	0.13(0.80)	-0.07(0.03)	0.52(0.50)	-0.26(0.05)	2.13(0.46)	-0.05(0.02)	0.00(0.25)
Asp283	-0.34(1.37)	-5.74(2.89)	0.76(1.59)	-12.95(2.5)	-1.82(0.52)	3.08(1.43)	-1.29(0.91)	2.29(2.08)
Asn284	-2.53(0.96)	-7.32(1.72)	-3.35(0.69)	-5.81(1.69)	-3.36(0.68)	-3.93(1.14)	-3.38(0.73)	-6.55(1.41)
Hse377	-2.18(1.24)	-12.19(1.8)	-1.87(1.52)	-13.75(2.4)	-1.42(1.51)	-12.19(2.1)	-2.20(1.47)	-12.24(2.4)
Thr378	-1.98(0.27)	-0.10(0.55)	-1.91(0.27)	0.21(0.55)	-2.25(0.31)	-0.43(0.58)	-2.10(0.26)	-0.00(0.55)
Lys574	-0.69(0.12)	-2.42(2.14)	-0.59(0.09)	0.62(1.80)	-0.69(0.11)	2.96(1.70)	-0.60(0.09)	-3.10(1.67)
Glu672	0.70(1.90)	-16.37(7.7)	-0.42(1.27)	-14.18(5.2)	0.77(1.50)	-19.09(4.38)	-0.15(1.30)	-12.23(5.1)
Gly135	-1.44(0.90)	-3.72(1.12)	-1.83(0.70)	-3.49(0.96)	-1.96(0.66)	-2.16(0.93)	-2.02(0.64)	-3.88(0.83)
Leu136	-3.40(0.53)	-0.74(0.29)	-4.59(0.53)	-2.10(0.58)	-4.07(0.54)	-0.67(0.38)	-4.74(0.52)	-1.48(0.49)

Note that the summations in the tables were done over the following residues: TIP3 Waters: 10, 61, 66, 76, 108, 111, 112, 139, 176, 200, 66*, 89*; Protein: Gly135, Leu136, Leu139, Asp283, Asn284, His377, Thr378, Val455, Asn484, Tyr573, Lys574, Glu672, Ala673, Ser674, Gly675, Thr676.

TABLE VII. Van der Waals and electrostatic interaction energies (kcal/mol) between important residues and the variable parts of the ligands: Hydan, O-hydan, Me-hydan and N-hydan. In the parentheses are the standard deviations. a=Wat066*, b=Wat108

	Hydan		O-hydan		Me-hydan		N-hydan	
Residue	elec	vdw	elec	vdw	elec	vdw	elec	vdw
Wat010	-1.69(2.30)	0.18(0.78)	-0.54(0.55)	-0.05(0.06)	1.62(0.67)	-0.18(0.06)	a-6.00(1.57)	a0.42(0.88)
	-3.54(1.77)	0.57(0.95)	b-0.59(0.21)	b-0.07(0.02)	-6.33(2.38)	1.05(1.19)	a-5.64(1.48)	a0.41(0.85)
Wat061	0.66(0.23)	-0.27(0.04)	1.57(0.34)	-0.26(0.05)	0.20(0.10)	-0.28(0.07)	0.12(0.16)	-0.28(0.06)
	0.64(0.21)	-0.26(0.04)	1.49(0.38)	-0.25(0.06)	0.19(0.12)	-0.30(0.07)	0.07(0.19)	-0.30(0.06)
Wat066			1.59(0.34)	-0.14(0.05)	0.13(0.05)	-0.04(0.03)	0.26(0.19)	-0.07(0.05)
			1.36(0.54)	-0.12(0.06)	0.17(0.06)	-0.06(0.04)	0.34(0.20)	-0.09(0.05)
Wat176	1.20(0.78)	-0.17(0.28)	-1.65(0.95)	-0.17(0.24)	-0.04(0.24)	-0.18(0.24)	0.07(0.46)	-0.23(0.23)
	0.74(1.00)	-0.19(0.23)	-1.96(0.90)	-0.06(0.41)	-0.07(0.24)	-0.18(0.23)	0.07(0.42)	-0.25(0.17)
Wat200	-0.12(0.61)	-0.21(0.06)	1.72(0.73)	-0.23(0.13)	-0.89(0.28)	0.10(0.46)	-1.76(1.07)	0.17(0.55)
	0.12(0.47)	-0.21(0.06)	0.70(1.11)	-0.11(0.14)	-0.88(0.26)	0.09(0.45)	-1.81(1.10)	0.15(0.57)
Wat089*	0.40(0.56)	-0.08(0.07)	0.53(0.41)	-0.04(0.02)	-4.42(3.27)	0.43(1.00)	0.07(0.25)	-0.04(0.02)
	a0.56(0.25)	a-0.03(0.01)	0.53(0.43)	-0.05(0.03)	2.06(0.44)	-0.20(0.04)	0.18(0.23)	-0.03(0.01)
Gly135	0.49(0.15)	-0.41(0.09)	0.92(0.24)	-0.57(0.18)	0.33(0.10)	-0.62(0.14)	0.34(0.17)	-0.65(0.14)
	0.53(0.16)	-0.44(0.10)	0.93(0.30)	-0.59(0.16)	0.30(0.10)	-0.62(0.15)	0.28(0.15)	-0.66(0.14)
Leu136	0.24(0.17)	-1.11(0.22)	-0.91(0.62)	-1.70(0.41)	0.26(0.14)	-1.72(0.29)	-0.37(0.35)	-1.83(0.38)
	0.20(0.15)	-1.00(0.24)	-0.96(0.57)	-1.70(0.38)	0.25(0.14)	-1.73(0.29)	-0.42(0.35)	-1.79(0.46)
Asp283	-13.17(2.36)	0.78(1.34)	-20.79(2.11)	1.60(1.66)	-2.86(0.53)	-0.99(0.54)	-7.08(1.81)	-0.32(1.01)
	-13.28(2.16)	0.75(1.36)	-20.86(2.00)	1.73(1.58)	-2.94(0.54)	-1.04(0.52)	-6.78(1.77)	-0.37(0.92)
Asn284	-2.23(1.05)	-1.38(0.46)	-2.29(1.12)	-1.98(0.38)	-0.09(0.60)	-2.08(0.31)	-0.96(0.68)	-2.08(0.28)
	-2.57(1.13)	-1.26(0.57)	-1.42(1.09)	-1.91(0.33)	0.05(0.56)	-2.03(0.30)	-1.51(0.78)	-2.16(0.25)
His377	-1.18(0.25)	-0.56(0.14)	-1.68(0.34)	-0.67(0.16)	-2.01(0.39)	-1.04(0.13)	-1.62(0.23)	-0.76(0.13)
	-1.11(0.25)	-0.52(0.13)	-1.85(0.30)	-0.68(0.15)	-1.92(0.39)	-1.00(0.14)	-1.46(0.26)	-0.76(0.14)
Thr378	-0.27(0.28)	-0.70(0.13)	-0.05(0.32)	-0.71(0.17)	-0.28(0.31)	-0.80(0.29)	-0.12(0.26)	-0.78(0.13)
	-0.26(0.26)	-0.69(0.14)	-0.06(0.30)	-0.69(0.15)	-0.22(0.32)	-0.82(0.24)	-0.18(0.25)	-0.76(0.16)
Lys574	1.83(0.27)	-0.07(0.02)	4.16(0.33)	-0.06(0.01)	1.66(0.20)	-0.08(0.02)	1.53(0.24)	-0.06(0.01)
	1.83(0.26)	-0.07(0.01)	4.15(0.30)	-0.06(0.01)	1.65(0.20)	-0.08(0.01)	1.51(0.27)	-0.07(0.01)
Glu672	-1.45(0.20)	-0.06(0.01)	-3.28(0.24)	-0.05(0.01)	-1.49(0.16)	-0.06(0.01)	-1.40(0.18)	-0.06(0.01)
	-1.46(0.20)	-0.06(0.01)	-3.34(0.23)	-0.06(0.01)	-1.53(0.16)	-0.06(0.01)	-1.41(0.20)	-0.06(0.01)
sum	-14.75()	-4.48()	-20.68()	-5.35()	-8.35()	-8.10()	-16.93()	-7.03()

II. FREE ENERGY CALCULATIONS

A. Methyl-hydan to N-hydan

In the forward calculation of free energy, the starting coordinates and velocities for the MNDA(the methyl-hydan and N-hydan hybrid) was taken from those of the methyl-hydan after 300 ps of simulation. After completing the forward direction, the system ran 100 ps more and the final configuration was used as the starting point for the backward simulation. Table VIII shows the comparison of the van der Waals energy and electrostatic energy at different λ (the percentage of N-hydan presence in the hybrid).

TABLE VIII. Derivatives as a function of λ for forward and backward simulations in protein and solution. We didn't perform the backward simulations for MNDA in solution.

λ	vdw(protein) forward(backward)	elec(protein) forward(backward)	vdw(solution) forward	elec(solution) forward
0.002	19.25(15.59)	0.22(0.97)	15.06	3.21
0.02	9.11(6.31)	-0.71(1.81)	6.99	2.51
0.1	4.01(3.37)	-0.92(-0.36)	2.57	1.62
0.2	1.63(1.29)	-1.78(-1.51)	1.13	0.61
0.3	0.31(0.06)	-1.92(-1.79)	0.21	0.12
0.4	-0.30(-0.64)	-2.83(-2.60)	-0.56	-0.17
0.5	-1.09(-1.65)	-2.74(-2.67)	-1.19	-0.71
0.6	-1.78(-2.34)	-3.39(-3.57)	-2.10	-1.13
0.7	-2.96(-3.02)	-4.24(-4.17)	-2.52	-1.60
0.8	-5.33(-4.84)	-4.62(-4.96)	-4.18	-2.49
0.9	-10.71(-9.31)	-4.01(-5.73)	-6.73	-2.83
0.98	-25.96(-29.95)	-6.27(-6.79)	-18.51	-4.80
0.998	-95.51(-96.15)	-6.36(-6.91)	-55.93	-4.97

TABLE IX. Free energy components by residues.

residue	vdw forward(backward)	elec forward(backward)
Asp283	-2.14(-2.31)	-2.80(-2.26)
His377	-0.22(-0.12)	0.47(0.34)
Asn284	-0.13(-0.12)	-0.16(-0.16)
Lys574	0.00(0.00)	-0.30(-0.33)
Glu672	0.00(0.00)	0.28(0.30)
Gly135	0.32(0.11)	0.13(0.16)
Leu136	-0.08(-0.08)	-0.33(-0.33)
W176	-0.72(-0.70)	0.03(-0.16)
W200	-0.43(-0.56)	-0.34(-0.35)
W61	0.19(0.26)	-0.02(-0.03)
W89*(W10)	0.07(-0.05)	-0.19(-0.19)
W139	-0.01(-0.01)	0.12(0.02)

TABLE X. The calculated free energy differences from the forward and backward simulations show that N-hydan is more favorable than Me-hydan both in protein and solution.

	in protein			in solution		
run	elec	vdw	total	elec	vdw	total
Me-ydan $\rightarrow$ N-hydan	-2.99	-3.68	-6.67	-0.77	-2.55	-3.33
N-hydan $\rightarrow$ Me-hydan	-2.97	-4.18	-7.15			
Average	-2.98	-3.93	-6.91			

B. Neutralized hydan to O-hydan

The neutralized hydan, denoted hereinafter as hydan0, refers to as an artificial spirohydantoin ligand whose C8, O8, N1, and HN1 hold zero charges. The purpose of such a neutralization procedure is to avoid possibly tremendous repulsive energy (the endpoint catastrophe) between the O9 of the hybrid and the OD1/OD2 of ASP283 at the hydan0 endpoint, where the HN1 atom forms a good hydrogen bond with the OD1 of ASP283 thus brings up considerable probability for the O9 to collide with the OD1, both of which have a relatively larger van der Waals radius.

The starting structure of the forward simulation, namely hydan0→O-hydan, was taken from an equilibrated structure in the hydan→hydan0 simulation (charges of C8, O8, N1, HN1 scaled with λ = 0.1), whereas the starting structure of the backward simulation, i.e. O-hydan→hydan0, was taken from the structure of the pure O-hydan at the end of a 300 ps of simulation.

TABLE XI. Derivatives as a function of λ (the ratio of the O-hydan presence in the hybrid HODA) for forward and backward simulations in protein for hydan0/O-hydan mutation, and in solution for hydan/O-hydan mutation.

λ	hydan0—O-hydan vdw(protein) forward(backward)	elec(protein) forward(backward)	hydan—O-hydan vdw(solution) forward(backward)	elec(solution) forward(backward)
0.002	109.78(91.17)	-0.27(-2.21)	140.95(135.16)	5.39(4.42)
0.02	25.02(21.77)	-0.76(-2.38)	29.87(30.15)	3.44(4.05)
0.1	6.09(4.93)	-3.33(-3.45)	7.24(7.71)	0.78(0.09)
0.2	2.11(1.77)	-4.76(-5.51)	3.41(3.20)	-0.96(-1.35)
0.3	1.45(0.77)	-6.95(-7.68)	1.36(1.70)	-2.73(-2.69)
0.4	0.92(-0.14)	-9.72(-8.77)	0.82(0.66)	-4.30(-4.11)
0.5	0.53(-0.15)	-11.22(-11.62)	-0.12(-0.43)	-5.93(-5.03)
0.6	0.15(-0.73)	-12.76(-13.94)	-0.68(-0.83)	-6.91(-6.77)
0.7	-0.11(-1.21)	-15.19(-14.35)	-1.04(-1.55)	-8.54(-7.58)
0.8	-0.94(-1.98)	-15.23(-21.67)	-1.88(-1.92)	-10.23(-9.85)
0.9	-2.59(-3.04)	-18.94(-21.64)	-3.25(-2.92)	-11.00(-11.98)
0.98	-5.67(-8.57)	-20.11(-23.08)	-6.58(-7.18)	-15.55(-13.60)
0.998	-8.33(-16.32)	-20.73(-23.44)	-11.67(-12.25)	-15.30(-14.77)

TABLE XII. Free energy components by residues.

residue	vdw forward(backward)	elec forward(backward)
Asp283	3.23(2.82)	-15.30(-14.32)
His377	0.25(0.06)	-2.63(-1.98)
Thr378	0.15(0.02)	-0.65(-0.26)
Asn284	-0.35(-0.60)	-1.84(-1.90)
Lys574	-0.01(-0.01)	4.19(3.74)
Glu672	-0.01(-0.00)	-3.34(-3.10)
Gly135	-0.33(-0.44)	1.04(0.88)
Leu136	-0.26(-0.44)	-0.26(-0.38)
W176	-0.02(2.20)	-0.16(-0.34)
W200	3.06(-0.01)	0.90(0.07)
W61	-0.35(-0.11)	1.57(1.25)
W89*	-0.13(-0.01)	0.84(0.88)

The free energy calculations show that the hydan0→O-hydan mutation is favored (Table XIII) both in the protein and in solution. The results reveal that the forward-backward hysteresis of the free energy difference (2.77 kcal/mol) is much greater in the protein than in solution.

TABLE XIII. The calculated free energy differences from the forward and backward simulations show that O-hydan is more favorable than hydan0 in protein and hydan in solution.

	in protein			in solution		
run	elec	vdw	total	elec	vdw	total
hydan0 $\rightarrow$ O-hydan	-10.80	3.40	-7.40	-5.56	3.88	-1.68
O-hydan $\rightarrow$ hydan0	-12.09	1.92	-10.17	-5.37	3.73	-1.64
Average	-11.45	2.66	-8.79	-5.47	3.81	-1.66

C. Hydan to O-hydan

The worry that the interaction between O9 of hydan and OD1/OD2 of Asp283 may lead to a huge van der Waals energy at the hydan endpoint which therefore cause considerable uncertainty to the free energy difference fades off after the simulation of hydan to O-hydan mutation was completed, with a prediction that the van der Waals contribution to the derivative at the worried endpoint is 193 kcal/mol compared with 110 kcal/mol in the case of hydan0 to O-hydan.

TABLE XIV. Derivatives as a function of λ (the ratio of the O-hydan presence in the hybrid HODA) for the forward and backward simulations in protein for the hydan/O-hydan mutation.

λ	vdw(forward/backward)	elec(forward/backward)
0.002	192.62(235.95)	4.65(6.91)
0.02	22.66(29.12)	2.18(4.26)
0.1	5.71(8.71)	-2.44(-6.63)
0.2	3.10(4.15)	-7.96(-7.82)
0.3	1.15(2.66)	-8.25(-7.31)
0.4	-0.13(0.22)	-8.42(-10.28)
0.5	-0.75(-0.29)	-10.21(-10.05)
0.6	-1.36(-1.32)	-10.25(-11.46)
0.7	-1.91(-1.84)	-10.31(-11.84)
0.8	-3.02(-2.79)	-10.82(-12.18)
0.9	-4.22(-4.02)	-11.71(-12.76)
0.98	-9.56(-7.29)	-10.96(-13.41)
0.998	-12.33(-10.25)	-11.81(-13.35)

TABLE XV. The calculated free energy differences from the forward and backward simulations show that O-hydan is more favorable than hydan both in protein and solution.

	in protein			in solution		
run	elec	vdw	total	elec	vdw	total
hydan $\rightarrow$ O-hydan	-9.34	5.41	-3.94	-5.56	3.88	-1.68
O-hydan $\rightarrow$ hydan	-8.39	3.55	-4.84	-5.37	3.73	-1.64
Average	-8.87	4.48	-4.39	-5.47	3.81	-1.66

TABLE XVI. Free energy components by residues: Ohydan-Hydan

residue	vdw forward(backward)	elec forward(backward)
Asp283	6.95(8.26)	-10.21(-10.21)
Asn284	-1.07(-0.74)	-0.22(0.12)
His377	-0.06(0.15)	-0.79(-1.47)
Thr378	-0.04(0.04)	0.31(0.22)
Tyr573	0.01(0.00)	-0.09(-0.07)
Lys574	-0.00(0.00)	1.90(2.29)
Glu672	0.00(0.00)	-1.66(-1.96)
Ser674	-0.00(-0.00)	0.16(0.24)
Gly675	-0.00(0.00)	0.18(0.24)
Gly135	-0.84(-0.74)	0.74(0.66)
Leu136	-0.16(-0.37)	-1.37(-1.41)
Wat10	-0.00(-0.03)	-0.16(-0.39)
Wat61	0.11(0.09)	0.45(0.36)
Wat66	-0.04(-0.00)	0.72(0.56)
Wat108	0.00(-0.00)	-0.13(-0.12)
Wat111	-0.00(-0.00)	0.16(-0.05)
Wat139	0.01(-0.03)	0.17(-0.03)
Wat176	0.65(1.17)	-1.34(-1.05)
Wat200	0.08(0.06)	1.95(2.06)
Wat89*	-0.00(-0.01)	0.06(0.23)

D. The free energy change due to the neutralization of the N1-HN1-C8-O8 group of hydan

Free energy calculation for a polarization reaction: hydan0→hydan has shown that the existence of the charges of the N1-HN1-C8-O8 group significantly helps to stabilize the ligand.

TABLE XVII. Derivatives as a function of λ (the scale factor on the charges of N1-HN1-C8-O8) for hydan0→hydan simulations in protein. Only electrostatic parts were calculated.

λ	elec
0.05	-3.70(-2.96)
0.1	-3.39(-3.44)
0.2	-4.07(-4.22)
0.3	-4.80(-5.12)
0.4	-5.24(-5.76)
0.5	-7.23(-6.34)
0.6	-9.17(-7.03)
0.7	-9.71(-9.67)
0.8	-12.37(-10.97)
0.9	-12.93(-13.45)
0.95	-15.01(-13.93)
integrand	-6.90(-6.55)

E. N-Hydan→O-Hydan

TABLE XVIII. Derivatives as a function of λ (the ratio of the O-hydan presence in the hybrid NODA) for the forward and backward simulations in protein for the N-hydan/O-hydan mutation.

λ	vdw(forward/backward)	elec(forward/backward)
0.002	24.37(23.23)	6.79(9.69)
0.02	11.16(12.98)	6.04(7.17)
0.1	3.89(4.89)	1.89(2.84)
0.2	2.31(3.18)	0.51(0.21)
0.3	1.57(2.02)	-1.08(-1.55)
0.4	0.94(1.16)	-3.64(-4.02)
0.5	0.86(0.59)	-5.19(-5.72)
0.6	0.35(0.19)	-7.08(-7.04)
0.7	-0.13(-0.59)	-8.49(-7.27)
0.8	-1.03(-1.72)	-9.09(-8.65)
0.9	-3.28(-4.64)	-11.29(-10.19)
0.98	-14.27(-20.23)	-13.40(-10.66)
0.998	-52.96(-85.40)	-12.55(-13.30)

TABLE XIX. The calculated free energy differences from the forward and backward simulations show that O-hydan is more favorable than N-hydan both in protein and solution(?).

	in protein			in solution		
run	elec	vdw	total	elec	vdw	total
N-hydan → O-hydan	-4.66	-0.10	-4.76			
O-hydan → N-hydan	-4.27	-0.91	-5.17			
Average	-4.47	-0.51	-4.97			

TABLE XX. Free energy components by residues: Ohydan-Hydan

residue	vdw forward(backward)	elec forward(backward)
Asp283	-0.81(-1.45)	-10.50(-7.97)
Asn284	0.23(0.17)	-0.75(-1.22)
His377	-0.04(-0.08)	-0.22(-0.26)
Thr378	0.02(0.08)	0.12(0.03)
Tyr573	-0.00(-0.00)	-0.20(-0.18)
Lys574	0.00(0.00)	2.69(2.28)
Glu672	-0.00(0.00)	-2.00(-1.72)
Ser674	0.00(0.00)	0.18(0.05)
Gly675	0.00(0.00)	0.16(0.16)
Gly135	0.20(0.05)	0.75(0.69)
Leu136	0.08(0.04)	-0.34(-0.02)
Wat10	0.02(0.00)	0.08(0.00)
Wat61	-0.04(-0.03)	1.67(1.40)
Wat66	0.00(0.00)	0.57(0.43)
Wat108	0.00(-0.00)	-0.16(-0.19)
Wat176	-0.06(-0.24)	-1.59(-1.38)
Wat200	-0.18(0.00)	2.35(-0.24)
Wat66*	0.12(-0.13)	0.38(0.81)

F. N-Hydan→Hydan0

TABLE XXI. Derivatives as a function of λ (the ratio of the N-hydan presence in the hybrid HNDA) for the forward and backward simulations in protein for the N-hydan/Hydan0 mutation.

λ	vdw(forward/backward)	elec(forward/backward)
0.0001	681.12()	-1.80()
0.0005	309.45()	-1.50()
0.001	224.63()	-1.50()
0.002	152.03(132.83)	-1.74(-0.68)
0.01	47.11( )	-0.70( )
0.02	27.23(27.97)	-0.97(-0.14)
0.06	7.15( )	-2.32( )
0.1	3.07(5.68)	-2.61(-1.36)
0.2	0.62(0.76)	-4.16(-2.35)
0.3	-1.04(-1.18)	-4.82(-3.88)
0.4	-1.83(-2.24)	-5.11(-4.93)
0.5	-2.47(-2.58)	-5.38(-6.01)
0.6	-2.98(-3.04)	-7.94(-6.45)
0.7	-3.26(-3.40)	-9.14(-7.32)
0.8	-4.12(-4.12)	-11.24(-9.97)
0.9	-5.19(-6.12)	-14.11(-12.40)
0.98	-8.42(-10.50)	-14.29(-14.20)
0.998	-14.48(-17.05)	-14.84(-15.66)
integration		
total -5.62(-5.99)	1.56(0.17)	-7.18(-6.16)

TABLE XXII. Free energy components by residues: Nydan-Hydan0

residue	vdw forward(backward)	elec forward(backward)
Asp283	3.30(3.10)	-5.12(-5.46)
Asn284	-0.71(-0.83)	-1.60(-0.93)
His377	-0.06(-0.00)	-1.39(-1.86)
Thr378	-0.07(-0.04)	-0.13(-0.33)
Tyr573	-0.02(-0.02)	-0.02(-0.00)
Lys574	-0.02(-0.02)	0.98(1.26)
Glu672	-0.01(-0.01)	-1.04(-1.27)
Ser674	-0.00(-0.00)	0.22(0.25)
Gly675	-0.00(-0.00)	0.13(0.16)
Gly135	-0.69(-0.74)	0.43(0.44)
Leu136	-0.49(-0.51)	-0.24(-0.36)
Wat10	-0.01(0.12)	-0.23(0.10)
Wat61	0.05(0.03)	-0.31(-0.30)
Wat66	-0.06(-0.04)	0.22(0.22)
Wat108	-0.00(-0.00)	-0.39(-0.21)
Wat176	1.59(1.65)	0.15(0.67)
Wat200	0.57(0.21)	-1.14(-1.35)
Wat66*	-0.11(-0.01)	-0.52(0.12)
direct	-5.49(tot)	-5.62(exact)
reverse	-4.99(tot)	-5.99(exact)

G. Hydan→N-hydan

The simulation result shows that Hydan is more stable than N-Hydan. This is seen from the following table that at the Hydan endpoint the van der Waals contribution to the free energy derivative is so large that the final integration ends up with a positive value which outnumbers the contribution from the negative electrostatic counterpart by 1.50 kcal/mol. This can be understood from the fact that the N-hydan mutation group is actually larger in size than the Hydan group.

TABLE XXIII. Derivatives as a function of λ (the ratio of the N-hydan presence in the hybrid HNDA) for the forward and backward simulations in protein for the Hydan/N-hydan mutation.

λ	vdw(forward/backward)	elec(forward/backward)
0.001	488.97()	3.33()
0.002	248.99()	1.80()
0.01	64.12()	2.35()
0.02	37.77()	1.78()
0.06	16.01()	1.29()
0.1	7.12()	-0.01()
0.2	3.20()	-1.22()
0.3	-0.22()	-1.80()
0.4	-1.16()	-3.30()
0.5	-1.08()	-2.74()
0.6	-2.21()	-3.66()
0.7	-3.04()	-5.32()
0.8	-4.25()	-5.47()
0.9	-5.76()	-4.65()
0.98	-9.73()	-7.18()
0.998	-16.11()	-6.88()
int.(1.50 total)	4.58()	-3.08()

TABLE XXIV. Free energy components by residues: Hydan→N-hydan

residue	vdw forward(backward)	elec forward(backward)
Asp283	5.97()	0.17()
Asn284	-0.79()	0.10()
His377	0.17()	-0.87()
Thr378	0.03()	-0.04()
Tyr573	-0.01()	0.08()
Lys574	-0.01()	-0.17()
Glu672	-0.00()	-0.07()
Ser674	-0.00()	0.07()
Gly675	-0.00()	0.05()
Gly135	-0.75()	-0.01()
Leu136	-0.58()	-0.74()
Wat10	0.11()	-0.21()
Wat61	-0.05()	-0.73()
Wat66	-0.03()	-0.06()
Wat108	-0.01()	-0.07()
Wat176	2.24()	0.82()
Wat200	0.26()	-0.74()
Wat66*	-0.00()	0.03()

III. LIGANDS IN SOLUTION

In this section, we discuss about the thermodynamical behaviors of the ligands in water. The radial distribution function of water molecules around a specific ligand atom is calculated and compared. The nonbonded interactions between the ligands and the aqueous environment are calculated as a function of the radius of hydration sphere

$$V_\alpha(R) = \sum_{|r_i - r_\alpha| < R} \phi_{i\alpha}(|r_i - r_\alpha|) \tag{1}$$

where ϕ is either van der Waals or electrostatic potential.

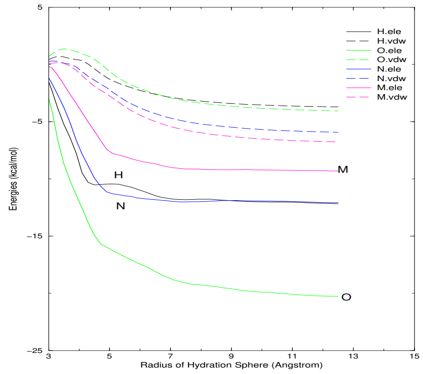

FIG. 6. Electrostatic and van der Waals energies of the four ligands as a function of the radius of the hydration sphere.

It is found that for all ligands, HO2, HO3, HO4 and HO6 of the sugar parts of the ligands form very good hydrogen bonds with waters—the first peaks of $g(r)$ sit at around 2 angstrom, with the peak values ranging from 1.2 to 1.5. The first peaks of $g(r)$ for HN2's of the five-member rings are found to be about half of those of HO*, which means in attracting water molecules HO* are doing much better than HN2(and HN1 in the case of hydan). This is due to the fact that the dipole moment of HO*-O* is bigger than that of HN*-N*?

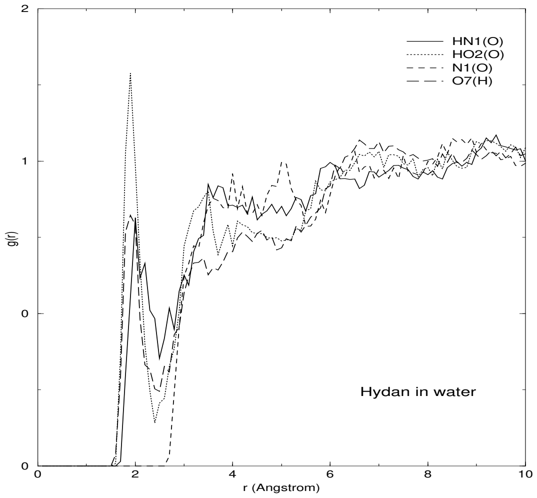

FIG. 7. Radial distribution functions of water around some atoms of HYDAN.

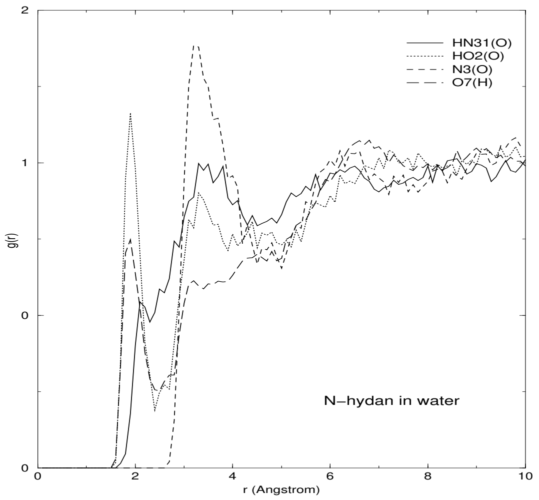

FIG. 8. Radial distribution functions of water around some atoms of N-HYDAN.

The $g(r)$'s for a methyl group is nothing interesting—there is just hard for it to form good hydrogen bonds with waters. Interestingly the $g(r)$'s for the NH_2 group show that there are no obvious peaks for $g_{HN1}(r)$ and $g_{HN2}(r)$ but there is one for $g_{N3}(r)$. This implies that there could be only one water which forms only one hydrogen bond with this group but the acceptor of the hydrogen bond switches frequently between HN1 and HN2. We have found that a simple Asn dipeptide exhibits similiar behavior for its NH2 group, despite of the fact that the peaks of $g_{HN1}(r)$ and $g_{HN2}(r)$ appear to be more observable but not pronounced either. I believe that this is a typical two attractor problem. A system with two attraction centers which are not far away from each other plausibly compete with each other to attract a water. But since both of them are linked to the N3 atom, it should be observed that $g_{N3}(r)$ has a significant peak.

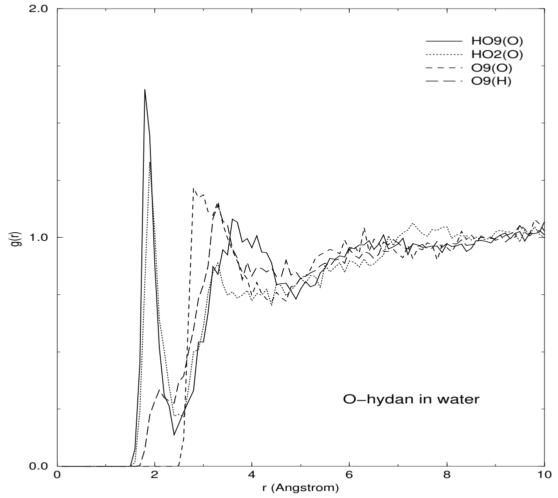

FIG. 9. Radial distribution functions of water around some atoms of O-HYDAN.

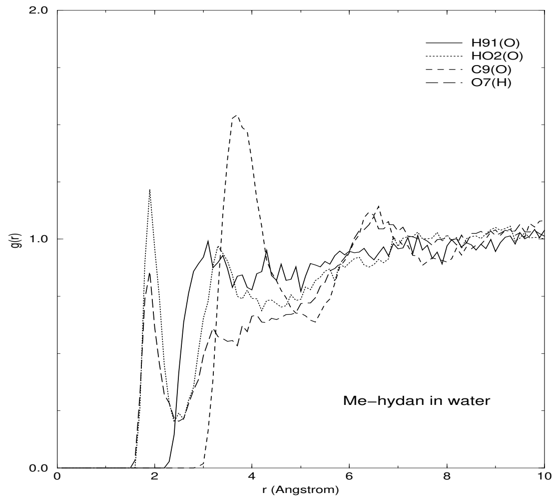

FIG. 10. Radial distribution functions of water around some atoms of Me-HYDAN.

IV. INFLUENCE OF WATERS 176 AND 200

It is found that water 176 plays an important role on stabilizing the ligand. In n-hydan and me-hydan, it stays closer to E88, whereas in hydan a position closer to D283 is more energetically favorable though the position closer to E88 seems still a local minimum. Therefore, it would be interesting to calculate the free energy profile of a path linking these two positions.

The calculation for a free energy profile is again based on the perturbation idea. A number of points along the path are selected, a reasonable harmonic constraint is imposed to confine the oxygen of the water to these points, thus the total free energy change due to the motion of water position from one endpoint to the other can be expressed by

$$\Delta A_{1N} = -\sum_{n=1}^{N-1} \ln \left\langle \exp\left[-\beta k_n \left(\mathbf{R}_{n+1}^c - \mathbf{R}_n^c\right) \cdot \left(\frac{\mathbf{R}_{n+1}^c + \mathbf{R}_n^c}{2} - \mathbf{r}_i\right)\right]\right\rangle_i \tag{2}$$

where $\mathbf{R}_n^c$ are the coordinates to which the water has to be constrained, k_n are the restraint constants, $\mathbf{r}_i$ are the coordinates of the water position during the simulation. The real free energy profile should be obtained by subtracting the contributions due to the constraints, which read

$$E_n^{corr} = -\ln \left\langle \exp\left[-\beta \frac{k_n}{2} \left(\mathbf{R}_n^c - \mathbf{r}_i\right)^2\right]\right\rangle_i \tag{3}$$

We first used the conjugate peak refinement (CPR) method to find a steepest descent path between the two minima, restrained the water around the points along this path with a force constant of 20 kcal/mol. the free energy profile without corrections is shown in Fig. 11.

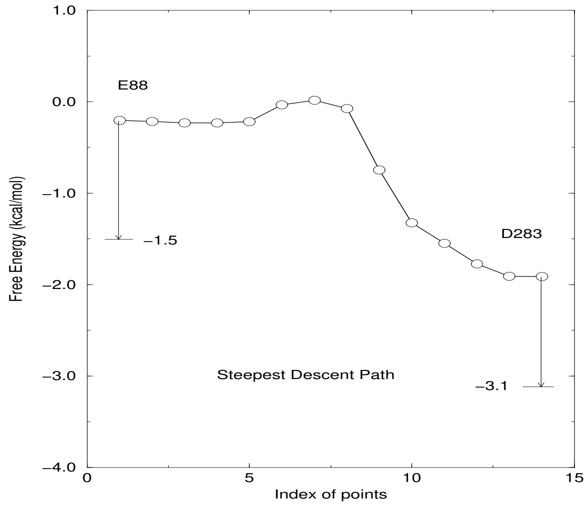

FIG. 11. Free energy profile along a steepest descent path obtained by the CPR method.

TABLE XXV. Data points for free enery profile and corrections. Force constants for the harmonic space restraints 20 kcal/mol.

Index	ΔA_{i1}	$E^{i}_{\rm corr}$	$\Delta A^{\rm corr}_{i1}$
1	-0.20	-1.30	-1.50
2	-0.21	-1.02	
3	-0.23	-1.18	
4	-0.23	-0.97	
5	-0.22	-1.25	
6	-0.03	-0.98	
7	0.02	-2.13	
8	-0.08	-1.68	
9	-0.75	-2.80	
10	-1.33	-3.50	
11	-1.55	-2.06	
12	-1.77	-1.72	
13	-1.91	-1.80	
14	-1.91	-1.16	-3.07

We also performed calculations where the distance of the water to the CD atom of E88 was constrained. The results are shown in Fig. 12.

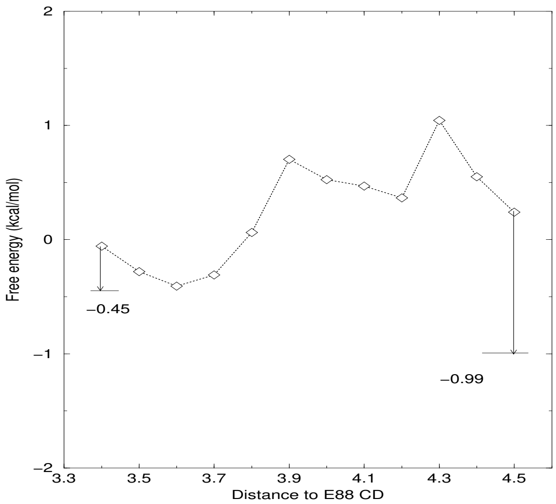

FIG. 12. Free energy profile by constraining the distance of the water to E88.

TABLE XXVI. Data points for free enery profile and corrections. Force constants for the harmonic distance restraints 40 kcal/mol

L_{OH2-CD}	ΔA_{i1}	E^{i}_{corr}	$\Delta A^{\mathrm{corr}}_{i1}$
4.5	0.24	-1.23	-0.99
4.4	0.55	-2.15	
4.3	1.04	-2.00	
4.2	0.37	-6.60	
4.1	0.47	-3.96	
4.0	0.53	-5.89	
3.9	0.70	-3.43	
3.8	0.06	-3.62	
3.7	-0.31	-4.95	
3.6	-0.41	-7.87	
3.5	-0.28	-8.24	
3.4	-0.06	-0.39	-0.45