Supplementary Material for "Contribution of Charged Groups to the Enthalpic Stabilization of the Folded States of Globular Proteins"

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Table 4S.

Enthalpic contribution of charged groups to protein folded state stability in RBNA. Nonbonded energy of group g, $< E^g_{nb} >$, (in kcal/mol); time average SASA, $< SA >$ (in Å^2); Exposure to solvent, SE, (in %); and enthalpic gain upon folding, ΔH^U_g, the change in enthalpy upon solvent reorganization, ΔH^U_h, and the total enthalpy change, $\Delta H^U = \Delta H^U_g + \Delta H^U_h$(in kcal/mol).

Table 5S.

Enthalpic contribution of charged groups to protein folded state stability in HLYSO. Nonbonded energy of group g ,$< E^g_{nb} >$, (in kcal/mol); time average SASA, $< SA >$ (in Å^2); exposure to solvent, SE, (in %); and enthalpic gain upon unfolding from environmental change, ΔH^U_g, solvent reorganization, ΔH^U_h, and the total change, $\Delta H^U = \Delta H^U_g + \Delta H^U_h$ (in kcal/mol).

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Table 7S.

Contribution of solvent "reorganization" to the change in system enthalpy upon protein unfolding: the average number of water molecules in the first, second and third hydration

shells, N_w^{1st}, N_w^{2nd}, N_w^{3rd}; the average nonbonded energy for each, $< E_{nb}^{1st} >$, $< E_{nb}^{2nd} >$ $< E_{nb}^{3rd} >$; the change in enthalpy of a water molecule, ΔH_w^{1st}, ΔH_w^{2nd}, ΔH_w^{3rd}; and the maximum change in solvent enthalpy upon complete charged group burial, ΔH_h^{max}. Energies are in kcal/mol. ΔH_w^{shell} is calculated as the difference between $< E_{nb}^{1st} >$ and the average nonbonded energy of a water molecule in the dipeptide solution, -9.2 kcal/mol.

Table 8S.

Enthalpy of unfolding - change in local environment of charged group upon protein unfolding, ΔH_g^U (kcal/mol), summed over charged groups of a given type as a function of exposure to solvent, SE, (in %). Group averages, $\overline{\Delta H_g^U}$, are averages over each charged group type in the indicated SE range, from 4 proteins. Global averages, $\widetilde{\Delta H_g^U}$, are averages over all charged groups in 4 proteins in the indicated SE range.

Table 9S.

Enthalpy of unfolding - contribution from solvent reorganization and structural collapse, ΔH_h^U (kcal/mol), summed over charged groups of a given type as a function of exposure to solvent, SE, (in %). Group averages, $\overline{\Delta H_h^U}$, are averages over each charged group type in the indicated SE range, from 4 proteins. Global averages, $\widetilde{\Delta H_h^U}$, are averages over all charged groups in 4 proteins in the indicated SE range.

Table 1: Enthalpy of folding and nonbonded energy for terminal CH_3 groups in apolar amino acids and their analog dipeptides

Group	$< E_{nb}^{g,dipep(g)} >$	$< E_{nb}^{g,prot} >$	ΔH_g^U
CH_3(alad)	+2.2	-	-
$C\delta_1(\delta_2)H_3$(vald)	-5.5	-	-
$C\gamma_1(\gamma_2)H_3$(leud)	-13.1	-	-
$C\gamma_2H_3$(iled)	+1.2	-	-
CH_3(ALA)		.2 - .8	.8 - 1.5
CH_3(VAL)		-5.5 - 6.6	.0 - .6
CH_3(ILE)		.8 - -2.6	+1.0 - 1.9
CH_3(LEU)		-14.2 - -15.2	.6 - 1.1
Average			$\propto$ +1.3

Table 2: The "unfolded state" nonbonded energy (in kcal/mol), and time average solvent accessible surface areas, $< SA >$ (in Å^2), of apolar and charged groups for solvated dipeptides and Ca^{2+} .

Group	$< E_{nb}^{g,dipep(g)} >$	$< SA^{g,dipep(g)} >$
CH_3(alad)	+2.25	77
$C_{\gamma 1}H_3$(vald)	-5.6	68
$C_{\gamma 2}H_3$(vald)	-5.2	63
$C_{\delta 1}H_3$(leud)	-13.2	71
$C_{\delta 2}H_3$(leud)	-13.2	70
$C_{\delta}H_3$(iled)	+1.23	77
$C_{\gamma 2}H_3$(iled)	-1.82	61
COO^-(aspd)	-201	97
COO^-(glud)	-184	98
Gu(argd)	-72	142
NH_3^+(lysd)	-86	59
Ca^{2+}	-606	122

Table 3: Enthalpic contribution of charged groups to protein folded state stability in TRPS. Nonbonded energy of group g, $< E^g_{nb} >$, (in kcal/mol), time average SASA, $< SA >$ (in Å^2), Exposure to solvent, SE, (in %), and enthalpic gain upon folding, ΔH^U_g, solvent reorganization, ΔH^U_h, and total enthalpy change, $\Delta H^U = \Delta H^U_g + \Delta H^U_h$ (in kcal/mol).

Group	$< E^g_{nb} >$	$< SA >$	SE	ΔH^U_g	ΔH^U_h	ΔH^U
COO^-(ASP84)	-210.5	0	0	+4.8	2.4	7.2
COO^-(ASP176)	-216.0	0	0	+7.5	2.4	9.9
COO^-(ASP171)	-191	2	2	-5	2.4	-2.6
COO^-(ASP145)	-227.5	52	52	+13.3	1.2	14.5
COO^-(ASP133)	-200.5	79	81	-.3	.5	.2
COO^-(ASP53)	-201.5	17	18	+.3	1.2	1.5
COO^-(GLU62)	-202.0	0	0	+9	1.7	10.7
COO^-(GLU52)	-208.0	0	0	+12	1.7	13.7
COO^-(GLU59)	-191	44	45	+3.5	.9	4.4
COO^-(GLU167)	-185.8	99	100	+.9	0.0	.9
Gu(ARG49)	-69.5	37	26	-1.3	1.4	.1
Gu(ARG99)	-74.8	94	67	+1.4	.6	2.0
NH_3^+(LYS43)	-82.5	18	31	-1.8	.5	-1.3
NH_3^+(LYS69)	-84.2	37	63	-.9	.4	-.5
NH_3^+(LYS89)	-91.1	27	46	+2.6	.6	3.2
NH_3^+(LYS91)	-79.6	40	68	-3.2	.4	-2.8
NH_3^+(LYS125)	-87.3	54	92	+.7	.1	.8
NH_3^+(LYS136)	-84.0	29	49	-1.0	.6	-.4
NH_3^+(LYS139)	-87.4	50	84	+.7	.2	.9
NH_3^+(LYS149)	-89	10	16	+1.5	.9	2.4
NH_3^+(LYS170)	-85.6	39	66	-.2	.4	.2
NH_3^+(LYS186)	-86.3	32	54	+.2	.5	.7
NH_3^+(LYS200)	-86.2	36	62	+.1	.4	.5
NH_3^+(LYS202)	-85.7	38	66	-.2	.4	.2
NH_3^+(LYS208)	-90.3	28	48	+2.2	.6	2.8
NH_3^+(LYS217)	-89.4	45	76	+1.7	.3	2.0

Table 4: Enthalpic contribution of charged groups to protein folded state stability in RBNA. Nonbonded energy of group g, $< E^g_{nb} >$, (in kcal/mol); time average SASA, $< SA >$ (in Å^2); Exposure to solvent, SE, (in %); and enthalpic gain upon folding, ΔH^U_g, the change in enthalpy upon solvent reorganization, ΔH^U_h, and the total enthalpy change, $\Delta H^U = \Delta H^U_g + \Delta H^U_h$(in kcal/mol)

Group	$< E^g_{nb} >$	$< SA >$	SE	ΔH^U_g	ΔH^U_h	ΔH^U
COO^-(ASP14)	-197.7	14	14	-1.7	2.1	.4
COO^-(ASP38)	-220.8	59	61	+9.9	.9	10.8
COO^-(ASP53)	-199.0	61	63	-1.0	.9	-.1
COO^-(ASP83)	-225.4	22	23	+12.2	1.8	14.0
COO^-(ASP121)	-212.5	8	8	+5.8	2.2	8.0
COO^-(GLU2)	-209.5	28	29	+12.8	1.2	14.0
COO^-(GLU9)	-191.0	82	84	+3.5	.3	3.8
COO^-(GLU49)	-177.9	41	42	-3.1	1.0	-2.1
COO^-(GLU86)	-192	38	38	+4	1.1	5.1
COO^-(GLU111)	-178.8	79	80	-2.6	.3	-2.3
Gu(ARG10)	-73.8	26	18	+.9	1.6	2.5
Gu(ARG33)	-75.8	33	23	+1.9	1.5	3.4
Gu(ARG39)	-71.7	101	72	-.2	.5	.3
Gu(ARG85)	-75.4	61	43	+1.7	1.1	2.8
NH_3^+(LYS1)	-82.6	58	100	-1.7	0.0	-1.7
NH_3^+(LYS7)	-81.6	9	15	-2.2	.9	-1.3
NH_3^+(LYS31)	-83.5	44	75	-1.3	.3	-1.0
NH_3^+(LYS37)	-91.3	29	50	+2.7	.6	3.3
NH_3^+(LYS41)	-77.9	15	25	-4.1	.8	-3.3
NH_3^+(LYS61)	-91.8	48	82	+2.9	.2	3.1
NH_3^+(LYS66)	-85.4	30	51	-.3	.5	.2
NH_3^+(LYS91)	-82.0	33	55	-2.0	.5	-1.5
NH_3^+(LYS98)	-84.3	56	95	-.9	.1	-.8
NH_3^+(LYS104)	-92.4	35	59	+3.7	.5	4.2

Table 5: Enthalpic contribution of charged groups to protein folded state stability in HLYSO. Nonbonded energy of group g ,$< E^g_{nb} >$, (in kcal/mol); time average SASA, $< SA >$ (in Å^2); exposure to solvent, SE, (in %); and enthalpic gain upon unfolding from environmental change, ΔH^U_g, solvent reorganization, ΔH^U_h, and the total change, $\Delta H^U = \Delta H^U_g + \Delta H^U_h$ (in kcal/mol).

Group	$< E^g_{nb} >$	$< SA >$	SE	ΔH^U_g	ΔH^U_h	ΔH^U
COO^-(ASP48)	-218.0	25	25	+8.5	1.8	10.3
COO^-(ASP66)	-216.0	1	0	+7.5	2.4	9.9
COO^-(ASP18)	-201.5	36	37	+.3	1.5	1.8
COO^-(ASP52)	-191.0	20	21	-5.0	1.9	-3.1
COO^-(ASP87)	-213.3	48	49	+6.2	1.2	7.4
COO^-(ASP101)	-212.5	55	56	+5.8	1.1	6.9
COO^-(ASP119)	-202.0	61	63	+.5	.9	1.4
COO^-(GLU7)	-181.2	61	62	-1.4	.6	-.8
COOH(GLU35)	-52.3	16	-	-		
Gu(ARG5)	-68.2	45	32	-1.9	1.6	-.3
Gu(ARG14)	-74.9	136	97	+1.5	.1	1.6
Gu(ARG21)	-72.3	101	72	+.2	.7	.9
Gu(ARG45)	-73.4	78	55	+.7	1.1	1.8
Gu(ARG61)	-78.2	27	19	+3.1	1.9	5.0
Gu(ARG68)	-69.6	128	91	-1.2	.2	-1.0
Gu(ARG73)	-68.4	91	65	-1.8	.8	-1.0
Gu(ARG112)	-74.3	73	52	+1.2	1.2	2.4
Gu(ARG114)	-74.2	97	69	+1.1	.7	1.8
Gu(ARG125)	-76.8	67	48	+2.4	1.2	3.6
Gu(ARG128)	-67.4	140	100	-2.2	0	-2.2
NH_3^+(LYS13)	-87.8	38	65	+.9	.4	1.3
NH_3^+(LYS33)	-82.3	41	70	-1.9	.3	-1.6
NH_3^+(LYS96)	-82.2	37	62	-1.9	.4	-1.5
NH_3^+(LYS97)	-89.0	38	65	+1.5	.4	1.9
NH_3^+(LYS116)	-88.1	35	59	+1.1	.5	1.6

Table 6: Enthalpic contribution of charged groups in ALACTA. Nonbonded energy of group g, $< E^g_{nb} >$, (in kcal/mol); time average SASA, $< SA >$ (in Å^2); exposure to solvent, SE, (in %); enthalpic gain upon folding from charged groups, ΔH^U_g, solvent reorganization, ΔH^U_h, and the net change in enthalpy, ΔH^U (in kcal/mol).

Group	$< E^g_{nb} >$	$< SA >$	SE	ΔH^U_g	ΔH^U_h	ΔH^U
COO^-(ASP15)	-210.2	63	64	+4.6	.9	5.5
COO^-(ASP38)	-203.5	39	40	+1.3	1.5	2.8
COO^-(ASP47)	-217.5	34	34	+8.3	1.6	9.9
COO^-(ASP65)	-189.0	84	86	-6.0	.3	-5.7
COO^-(ASP79)	-204.0	32	33	+1.5	1.6	3.1
COO^-(ASP83)	-218.4	13	13	+8.7	2.1	10.8
COO^-(ASP84)	-189.4	63	64	-5.8	.9	-4.9
COO^-(ASP85)	-192.2	44	64	-4.4	.9	-3.5
COO^-(ASP88)	-218.0	16	17	+8.5	2.0	10.5
COO^-(ASP89)	-213.3	0	0	+6.2	2.4	8.6
COO^-(ASP117)	-201.4	52	53	+.2	1.1	1.3
COO^-(GLU2)	-181.0	96	98	-1.5	0.0	-1.5
COO^-(GLU8)	-182.9	44	45	-.6	.9	.3
COO^-(GLU12)	-180.8	31	32	-1.6	1.2	-.4
COO^-(GLU26)	-188.6	36	36	+2.3	1.1	3.3
COO^-(GLU50)	-189.5	50	51	+2.8	.8	3.6
COO^-(GLU114)	-181.4	84	86	-1.3	.2	-1.1
COO^-(GLU122)	-178	83	85	-3.0	.3	-2.7
Gu(ARG11)	-82.4	141	100	+5.2	0.0	5.2
NH_3^+(LYS6)	-82.7	40	68	-1.7	.4	-1.3
NH_3^+(LYS14)	-91.5	37	62	+2.8	.4	3.2
NH_3^+(LYS17)	-89.2	47	80	+1.6	.2	1.8
NH_3^+(LYS59)	-89.8	33	56	+1.9	.5	2.4
NH_3^+(LYS63)	-84.0	2.6	4	-1.0	1.1	.1
NH_3^+(LYS80)	-88.0	40	68	+2.0	.4	2.4
NH_3^+(LYS94)	-82.6	36	60	-1.7	.4	-1.3
NH_3^+(LYS95)	-78	16	27	-4.0	.8	-3.2
NH_3^+(LYS109)	-81.4	50	85	-2.3	.2	-2.1
NH_3^+(LYS115)	-82.6	33	55	-1.7	.5	-1.2
NH_3^+(LYS123)	-80.1	37	62	-3.0	.4	-2.6

Table 7: Contribution of solvent "reorganization" to the change in system enthalpy upon protein unfolding: the average number of water molecules in the first, second and third hydration shells, N_w^1, N_w^2, N_w^3; the average nonbonded energy for each, E_{nb}^1, E_{nb}^2 E_{nb}^3; the change in enthalpy of a water molecule for each hydration shell, ΔH_w^1, ΔH_w^2, ΔH_w^3
; and the maximum change in solvent enthalpy upon complete charged group burial, ΔH_h^{max}. Energies are in kcal/mol. For each shell, ΔH_w^{sh} is calculated as the difference between E_{nb}^{sh} and the average nonbonded energy of a water molecule in the dipeptide solution, -9.2 kcal/mol.

Group	N_w^1	E_{nb}^1	ΔH_w^1	N_w^2	E_{nb}^2	ΔH_w^2	N_w^3	E_{nb}^3	ΔH_w^3	ΔH_h^{max}
glud	4.5	-12.3	-3.1	17.0	-8.6	.6	27	-9.0	.2	1.7
aspd	4.1	-11.7	-2.5	13.4	-8.7	.5	27	-9.0	.2	2.4
lysd	4.1	-11.5	-2.3	15.6	-8.7	.5	28	-9.1	.1	1.1
argd	4.9	-10.3	-1.1	18.1	-8.8	.4	27.8	-9.2	0.0	1.9
vald	5.7	-8.7	.5	9.8	-9.7	-.5	22	-9.0	.2	2.4

Table 8: Enthalpy of unfolding - change in local environment of charged group upon protein unfolding, ΔH_g^U (kcal/mol), summed over charged groups of a given type as a function of exposure to solvent, SE, (in %). Group averages, $\overline{\Delta H_g^U}$, are averages over each charged group type in the indicated SE range, from 4 proteins. Global averages, $\widetilde{\Delta H_g^U}$, are averages over all charged groups in 4 proteins in the indicated SE range.

Group	Protein	ΔH_g^U					
		0-100	0-20	20-40	40-60	60-80	80-100
	TRPS	+20.6	+7.6	0	+13.3	0	-.3
g=	RBNA	+25.2	+4.1	+12.2	0	+8.9	0
COO^-	HLYSO	+23.0	+7.5	+3.5	+12.0	0	0
(ASP)	ALACTA	+23.1	+23.4	+11.1	+.2	-5.6	-6
	$\sum \Delta H_g^U$	+91.9	+42.6	+26.8	+25.5	+3.3	-6.3
	$\sum N^g$	29	10	7	4	6	2
	$\overline{\Delta H_g^U}$	+3.2	+4.3	+3.8	+6.4	.6	-3.2
	TRPS	+25.4	+21	0	+3.5	0	+.9
g=	RBNA	+14.6	0	+16.8	-3.1	-2.6	+3.5
COO^-	HLYSO	-1.4	0	0	0	-1.4	0
(GLU)	ALACTA	-2.9	0	+.7	+2.2	0	-5.8
	$\sum \Delta H_g^U$	+35.7	+21.0	+17.5	+2.6	-4.0	-1.4
	$\sum N^g$	17	2	4	4	2	5
	$\overline{\Delta H_g^U}$	+2.1	+10.5	+4.4	+.7	-2.0	-.3
	TRPS	+.1	0	-1.3	0	+1.4	0
g=	RBNA	+4.3	+.9	+1.9	+1.7	-.2	0
Gu(ARG)	HLYSO	+3.1	+3.1	-1.9	+4.3	-.5	-1.9
	ALACTA	+5.2	0	0	0	0	+5.2
	$\sum \Delta H_g^U$	+12.7	+4.0	-1.3	+6	+.7	+4.3
	$\sum N^g$	18	2	3	4	5	4
	$\overline{\Delta H_g^U}$	+.7	+2	-.4	+1.5	+.1	+1.1
	TRPS	+2.4	+1.5	-1.8	+4.0	-2.7	+1.4
g=	RBNA	-3.8	-2.2	-4.1	+3.6	-1.3	+.3
NH_3^+	HLYSO	-.3	0	0	+1.1	-1.4	0
(LYS)	ALACTA	-7.1	-1.0	-4.0	-1.5	+1.7	-2.3
	$\sum \Delta H_g^U$	-8.8	-1.7	-9.9	+6.6	-3.7	-.1
	$\sum N^g$	40	3	3	11	17	6
	$\overline{\Delta H_g^U}$	-.2	-.6	-3.3	+.7	-.2	-.1
All	$\sum\sum \Delta H_g^U$	131.5	65.9	33.1	38.1	-3.7	-3.5
All	$\sum\sum N^g$	104	1117	17	23	30	17
Ave All	$\widetilde{\Delta H_g^U}$	1.26	+3.9	1.9	+1.7	-.1	-.2

Table 9: Enthalpy of unfolding - contribution from solvent reorganization and structural collapse, ΔH_h^U (kcal/mol), summed over charged groups of a given type as a function of exposure to solvent, SE, (in %). Group averages, $\overline{\Delta H_h^U}$, are averages over each charged group type in the indicated SE range, from 4 proteins. Global averages, $\widetilde{\Delta H_h^U}$, are averages over all charged groups in 4 proteins in the indicated SE range.

Group	Protein	ΔH_h^U					
		0-100	0-20	20-40	40-60	60-80	80-100
	TRPS	10.1	8.4	0	1.2	0	.5
g=	RBNA	7.9	4.3	1.8	.9	.9	0
COO^-	HLYSO	10.8	2.4	5.2	2.3	0.9	0
(ASP)	ALACTA	15.3	6.5	4.7	1.1	2.7	.3
	$\sum \Delta H_h^U$	43.1	21.6	11.7	5.5	+3.5	.8
	$\sum N^g$	29	10	7	4	6	2
	$\overline{\Delta H_h^U}$	1.48	2.2	1.7	1.4	.6	.4
	TRPS	4.3	3.4	0	.9	0	0
g=	RBNA	3.9	0	2.3	1.0	.3	.3
COO^-	HLYSO	.6	0	0	0	.6	0
(GLU)	ALACTA	4.5	0	2.3	1.7	0	.5
	$\sum \Delta H_h^U$	13.1	3.4	4.6	3.6	1.7	.8
	$\sum N^g$	17	2	4	4	2	5
	$\overline{\Delta H_h^U}$	.8	1.7	1.2	.8	.9	.2
	TRPS	2.0	0	1.4	0	.6	0
g=	RBNA	4.7	1.6	1.5	1.1	.5	0
Gu(ARG)	HLYSO	9.5	1.9	1.6	3.5	2.2	.3
	ALACTA	0	0	0	0	0	0
	$\sum \Delta H_h^U$	16.2	3.5	4.5	4.6	3.3	.3
	$\sum N^g$	18	2	3	4	5	4
	$\overline{\Delta H_h^U}$	.9	1.8	1.5	1.2	.7	.1
	TRPS	6.3	.9	.5	2.3	2.5	.3
g=	RBNA	4.4	.9	.8	2.1	.3	+.3
NH_3^+	HLYSO	2.0	0	0	.5	1.5	0
(LYS)	ALACTA	5.3	1.1	.8	1.4	1.8	.2
	$\sum \Delta H_h^U$	18.2	2.9	2.1	6.3	5.3	.8
	$\sum N^g$	40	3	3	11	17	6
	$\overline{\Delta H_h^U}$	.5	1.0	.7	.6	.3	.1
All	$\sum \Delta H_h^U$	90.6	31.4	22.9	20.0	13.8	2.7
All	$\sum N^g$	104	17	17	23	30	17
All Ave	$\widetilde{\Delta H^U}$	.9	1.8	1.3	.9	.03	.16