Appendix I. MCCE calculated fractional residue ionization at pH 7

	bR					early M				late M		
	1C8R	1C8R (Explicit)	1C3W	1KG9	1M0M-1	1DZE	1DZE (Explicit)	1KG8	1M0M-2	1C8S	1C8S (Explicit)	1F4Z
Arg7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glu9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys30	1.00	1.00	1.00	0.96	**0.89**	0.99	0.99	0.96	0.90	1.00	1.00	1.00
Asp36	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Asp38	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys40	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys41	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glu74	0.99	0.99	0.99	0.98	0.99	1.00	1.00	**0.81**	0.99	0.97	**0.89**	0.99
Arg82	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Asp85	**0.98**	**0.83**	**0.93**	**1.00**	**0.73**	**0.67**	**0.73**	**0.14**	**0.39**	**0.07**	**0.01**	**0.00**
Asp96	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Asp102	0.98	0.97	0.99	0.98	0.98	0.96	0.97	0.98	0.99	0.99	1.00	0.98
Asp104	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Asp115	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Lys129	1.00	1.00	1.00	1.00	1.00	**0.86**	**0.86**	1.00	1.00	1.00	0.99	1.00
Arg134	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys159	N.A.	N.A.	N.A.	N.A.	N.A.	1.00	1.00	N.A.	N.A.	N.A.	N.A.	N.A.
Glu161	N.A.	N.A.	N.A.	N.A.	N.A.	1.00	1.00	N.A.	N.A.	N.A.	N.A.	N.A.
Arg164	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	1.00	N.A.	N.A.	1.00
Glu166	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	1.00	N.A.	N.A.	1.00
Lys172	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	N.A.	1.00
Arg175	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	N.A.	1.00
Glu194	**0.00**	**0.00**	**0.01**	**0.17**	**0.01**	**0.00**	**0.01**	**0.00**	**0.00**	**0.51**	**0.64**	**0.79**
Glu204	**1.00**	**1.00**	**1.00**	**0.83**	**0.99**	**1.00**	**0.99**	**1.00**	**1.00**	**0.65**	**0.74**	**0.21**
Asp212	**0.97**	**1.00**	**0.90**	**0.99**	**0.90**	**0.43**	**0.33**	**0.91**	**0.92**	**0.92**	**0.95**	**1.00**
SB216	**0.95**	**0.84**	**0.83**	**0.99**	**0.62**	**0.12**	**0.06**	**0.05**	**0.32**	**0.13**	**0.14**	**0.00**
Arg225	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	N.A.	1.00
Arg227	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N.A.	N.A.	1.00

All structures are calculated by filling cavities with continuum water (dielectric constant 80). (Explicit) 1C8R, 1DZE and 1C8S in bR, early and late M respectively also calculated with explicit waters in cavities. The central and release cluster residues are highlighted, as are all cases where less than 90% of the residue is ionized. No Tyr are ionized. *N.A.* Residues not resolved in the crystal structures.

The following ionizable residues are not resolved in the crystal structures:

All structures: E232, E234, E237 and D242 in C terminus

All structures except 1DZE: K159 and E161 in E-F loop

1KG8: Arg164 and Glu166 in E-F loop

1C8S: Arg164, Glu166, Lys172 and Arg175 in E-F loop and Arg 225 and 227 in C terminus

Appendix II. Per residue mean field interactions with the central and proton release cluster residues in calculations with explicit waters in cavities at pH 7.

	SB			Asp85			Asp212			Glu194			Glu204		
	bR (1C8R)	early M (1DZE)	late M (1C8S)	bR (1C8R)	early M (1DZE)	late M (1C8S)	bR (1C8R)	early M (1DZE)	late M (1C8S)	bR (1C8R)	early M (1DZE)	late M (1C8S)	bR (1C8R)	early M (1DZE)	late M (1C8S)
Thr5	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-
Arg7	0.3	0.1	0.3	-0.5	-0.1	-0.6	-0.5	-0.1	-0.5	-0.5	-0.1	-0.5	-0.6	-0.1	-0.5
Glu9	-0.5	-0.5	-0.6	0.7	0.6	0.8	0.8	**1.0**	**1.0**	**1.2**	**1.1**	**1.2**	**1.5**	**1.5**	**1.6**
Thr17	-	-	-	-	-	-	-0.1	-0.1	-0.1	-	-	-	-0.1	-0.1	-
Thr24	0.1	0.1	-	-0.1	-0.1	-	-0.1	-0.1	-0.1	-	-	-	-	-	-
Tyr26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lys30	0.2	0.2	0.2	-0.2	-0.2	-0.3	-0.2	-0.2	-0.2	-	-0.1	-0.1	-	-	-0.1
Ser35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asp36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asp38	-0.1	-	-0.1	0.1	0.1	0.1	0.1	-	0.1	-	-	-	-	-	-
Lys40	-	-	-	-0.1	-0.1	-0.1	-	-	-	-	-	-	-	-	-
Lys41	0.1	0.1	0.1	-0.1	-0.1	-0.2	-0.1	-0.1	-0.1	-	-	-	-	-	-
Tyr43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thr46	-0.1	-0.1	-0.1	-	-	-	0.1	-	0.1	-	-	-	-	-	-
Thr47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thr55	0.1	0.1	0.1	-0.2	-0.3	-0.3	-0.1	-0.1	-0.1	-	-	-	-	-	-
Tyr57	0.4	0.1	0.3	-0.6	-0.1	-0.6	**-2.1**	-1.8	-1.4	0.1	-	-	0.1	-	-
Ser59	0.1	0.1	0.1	-0.3	-0.3	-0.3	-0.2	-0.2	-0.2	-	-	-	-0.1	-	-
Tyr64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thr67	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	-	0.1	-
Glu74	-	-0.1	-0.1	-	-	-	-	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.3
Gln75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asn76	-	-	-	-	-	-	-	-	-	-0.2	-0.1	-0.1	-	-	-
Tyr79	-	-	-	-	-	-	-	-	-	-0.3	-0.2	-0.2	-0.2	-0.2	-0.2
Arg82	**3.5**	**3.0**	**2.7**	**-4.6**	**-3.9**	**-3.9**	**-6.3**	**-6.4**	**-5.2**	**-6.3**	**-8.5**	**-7.1**	**-6.2**	**-7.8**	**-6.8**
Tyr83	0.1	-0.1	-0.2	-0.1	-	-	-0.1	-	0.1	-0.2	-1.0	-1.8	-0.1	-0.2	-0.2
Asp85	**-6.6**	**-4.9**	-0.1	*x*	*x*	*x*	**6.3**	**6.6**	-0.2	**1.5**	**1.5**	-0.1	**1.4**	**1.2**	-0.1
Thr89	0.7	-	0.5	**-1.9**	**-2.1**	-0.7	-0.4	-0.2	-0.4	-	-	-	-	-	-
Thr90	0.1	-	0.1	-0.4	-0.3	-0.4	-0.1	-0.1	-0.2	-	-	-	-	-	-
Asp96	0.1	-	-	-0.1	-	-0.1	-0.1	-	-0.1	-	-	-	-	-	-
Asp102	-	-	-0.1	-	-	0.1	-	-	-	-	-	-	-	-	-
Asp104	-	-	-0.1	-	-	0.1	-	-	-	-	-	-	-	-	-
Gln105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thr107	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asp115	-	-	-	-0.1	-0.1	-0.1	-	-0.1	-0.1	-	-	-	-	-	-
Thr121	-	-	-	-0.1	-	-	-	-	-	-	-	-	-	-	-

Thr128	-	0.1	0.1	-	-0.1	-0.1	-	-0.1	-0.1	-0.1	-0.2	-0.2	-	-0.1	-0.1
Lys129	0.1	0.1	0.1	-0.1	-0.1	-0.1	-	-0.1	-0.1	-0.1	-0.3	-0.3	-0.1	-0.1	-0.2
Tyr131	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ser132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tyr133	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arg134	0.9	**1.0**	**1.0**	-0.6	-0.6	-0.6	-0.6	-0.8	-0.8	**-4.0**	**-3.9**	**-4.0**	**-2.2**	**-2.3**	**-2.1**
Ser141	-0.2	-0.3	-	-	-	-	-	-	-	-	0.1	-0.1	-	0.1	-
Thr142	0.1	-	0.1	-	-	-	-	-	-	-	-0.1	-0.1	-	-	-
Tyr147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tyr150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thr157	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*
Ser158	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*
Lys159	*N.A.*	0.1	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*
Glu161	*N.A.*	-0.1	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*	*N.A.*	-	*N.A.*
Ser162	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Arg164	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Glu166	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Ser169	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Thr170	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Lys172	-	0.1	*N.A.*	-	-0.1	*N.A.*	-	-0.1	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Arg175	0.5	0.5	*N.A.*	-0.3	-0.3	*N.A.*	-0.3	-0.4	*N.A.*	-0.1	-0.2	*N.A.*	-0.1	-0.1	*N.A.*
Asn176	-0.1	-	-	0.1	-	-	0.1	-	-	-	-	-	-	-	-
Thr178	-0.1	-0.1	-	-	0.1	-	0.1	0.1	-	-	-	-	-	-	-
Ser183	0.1	0.1	-	-	-	-	-0.1	-0.1	-0.1	-	-	-	-	-	-
Tyr185	0.2	-	-0.1	-0.3	-0.2	-0.2	**-5.0**	**-1.2**	**-6.2**	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1
Ser193	0.1	0.1	0.1	-0.1	-0.1	-0.1	-0.1	-0.2	-0.2	-0.4	-0.9	-0.7	**-2.6**	**-2.5**	**-2.1**
Glu194	0.1	-0.2	**-1.4**	-	0.2	**1.2**	-0.1	0.2	**1.4**	*x*	*s*	*x*	**-7.3**	**-4.5**	**4.3**
Asn202	-	-	-0.1	-	-0.1	0.1	0.1	-0.1	0.2	-	-0.1	0.1	-	-0.1	0.2
Glu204	**-1.9**	**-1.7**	-0.9	**1.6**	**1.5**	0.9	**2.4**	**2.6**	**1.3**	**14.0**	**13.3**	**5.9**	*x*	*x*	*x*
Thr205	-0.1	-0.1	-0.1	0.1	0.1	0.1	0.2	0.2	0.2	-0.1	-	0.1	-0.3	-0.4	-
Asp212	**-8.9**	**-1.9**	**-6.8**	**8.8**	**1.5**	**8.4**	*x*	*x*	*x*	**2.6**	0.8	2.6	**2.7**	0.8	2.7
Ser214	-	0.1	0.2	-	-0.1	-0.2	-	-0.3	-0.5	-	-	-0.1	-	-0.1	-0.1
SB(216)	*x*	*x*	*x*	**-6.2**	**-1.3**	-0.9	**-6.5**	**-1.0**	**-1.5**	**-1.9**	-0.5	-0.4	**-1.8**	-0.3	-0.3
Arg225	0.1	0.2	*N.A.*	-0.1	-0.2	*N.A.*	-0.1	-0.2	*N.A.*	-	-0.1	*N.A.*	-	-0.1	*N.A.*
Ser226	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*
Arg227	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*	-	-	*N.A.*

Mean field interactions of all ionizable and polar side chains with the central and proton release cluster residues. All energies in ΔpK_a units. 1 pK_a unit = 1.36 kcal/mol. Cluster residues in bold. The mean-field interaction energy of a residue with a cluster residue (i) is $\sum r_k \Delta G_{ik}$ where r_k is the occupancy of the kth conformer of the residue in full Monte Carlo sampling and ΔG_{jk} the interaction of the cluster residue conformer (i) with conformer k. r_k can be pH dependent. The difference in interaction energy of the external residue with the ionized and neutral cluster residue are given. Values <0.1 ΔpK_a unit are not shown. Values >±1 ΔpK unit in bold. Those >±3 ΔpK units bold and underlined. *N.A.* residues are missing from the structure. *x:* cluster residue interaction with itself.

APPENDIX III. Terms contributing to cluster microstate energies in the reduced model of the central cluster.

A. Interactions with the rest of the protein and the surrounding water.

	Waters in cavities	Residue	Solution pK_a	Reaction field energy loss	Backbone Interaction	MFE'	pK'
bR	continuum	SB	7.00	4.10	2.09	4.80	-3.98
		Asp85	4.75	8.69	-3.77	-8.40	1.27
		Asp212	4.75	9.02	-2.49	-10.30	0.97
	explicit	SB	7.00	4.30	2.13	5.60	-5.01
		Asp85	4.75	9.96	-3.44	-10.00	1.27
		Asp212	4.75	9.96	-2.52	-16.50	-4.31
early M	continuum	SB	7.00	4.10	1.25	3.10	-1.45
		Asp85	4.75	9.11	-4.46	-6.20	3.20
		Asp212	4.75	9.33	-1.77	-8.10	4.21
	explicit	SB	7.00	4.25	1.31	3.60	-2.17
		Asp85	4.75	9.94	-2.75	-9.70	2.23
		Asp212	4.75	9.96	-1.75	-8.80	4.16
late M	continuum	SB	7.00	3.99	1.74	2.60	-1.33
		Asp85	4.75	8.59	-2.55	-4.70	6.09
		Asp212	4.75	7.87	-1.62	-11.30	-0.30
	explicit	SB	7.00	4.34	2.11	2.80	-2.26
		Asp85	4.75	9.84	-3.49	-4.60	6.50
		Asp212	4.75	10.01	-2.64	-13.20	-1.08

B. Pair-wise interactions between ionized and neutral conformers of the residues in the central cluster.

	Waters in cavities		SB^0	SB^+	$Asp212^0$	$Asp212^-$
bR	continuum	SB^0	-	-	-0.27	1.02
		SB^+	-	-	0.06	-7.72
		$Asp85^0$	-0.32	0.31	0.00	-0.47
		$Asp85^-$	0.77	-7.35	0.04	8.11
	explicit	SB^0	-	-	-0.58	0.96
		SB^+	-	-	0.35	-7.94
		$Asp85^0$	0.20	-0.26	0.13	0.88
		$Asp85^-$	0.78	-7.49	0.38	8.27
early M	continuum	SB^0	-	-	-0.14	-0.69
		SB^+	-	-	0.13	-7.13
		$Asp85^0$	-0.08	0.09	-0.02	-0.47
		$Asp85^-$	-0.77	-6.56	0.36	8.13
	explicit	SB^0	-	-	-0.20	-0.68
		SB^+	-	-	-0.85	-7.25
		$Asp85^0$	-0.06	0.21	0.04	-0.43
		$Asp85^-$	-0.81	-6.95	0.15	8.36
late M	continuum	SB^0	-	-	-0.58	-1.26
		SB^+	-	-	-0.26	-7.56
		$Asp85^0$	-0.24	-0.47	0.08	0.77
		$Asp85^-$	-0.44	-5.94	0.54	7.40
	explicit	SB^0	-	-	-0.56	-1.31
		SB^+	-	-	-0.24	-8.28
		$Asp85^0$	-0.02	-0.21	-0.21	-0.95
		$Asp85^-$	-0.41	-6.35	0.41	7.58

C. Energies and occupancies of the 8 microstates of central cluster in the reduced model.

Continuum Water in Internal Cavities									
Microstate Ionization			Net charge	BR		Early M		Late M	
				Energy	Occupancy	Energy	Occupancy	Energy	Occupancy
SB^0	$D85^0$	$D212^0$	0	-0.6	0.000	-0.2	0.000	-0.7	0.000
		$D212^-$	–1	-5.8	0.011	-4.0	**0.279**	-8.0	**0.976**
	$D85^-$	$D212^0$	–1	-5.2	0.003	-4.3	**0.587**	-1.4	0.000
		$D212^-$	–2	-1.9	0.000	0.1	0.000	-2.5	0.000
SB^+	$D85^0$	$D212^0$	1	11.3	0.000	8.7	0.000	7.7	0.000
		$D212^-$	0	-3.0	0.000	-1.8	0.002	-6.2	0.016
	$D85^-$	$D212^0$	0	-2.0	0.000	-1.4	0.001	1.8	0.000
		$D212^-$	–1	-7.8	**0.986**	-3.7	**0.132**	-6.0	0.009

Explicit Water in Internal Cavities									
SB^0	$D85^0$	$D212^0$	0	-0.3	0.000	-0.2	0.000	-0.8	0.000
		$D212^-$	–1	-9.3	0.001	-4.0	0.022	-10.4	**0.992**
	$D85^-$	$D212^0$	–1	-5.2	0.000	-5.6	**0.932**	-1.1	0.000
		$D212^-$	–2	-7.1	0.000	-0.7	0.000	-2.8	0.000
SB^+	$D85^0$	$D212^0$	1	12.3	0.000	8.6	0.000	8.6	0.000
		$D212^-$	0	-6.7	0.000	-1.1	0.000	-8.3	0.008
	$D85^-$	$D212^0$	0	-0.6	0.000	-3.2	0.004	2.5	0.000
		$D212^-$	–1	-12.3	**0.999**	-4.2	0.042	-6.4	0.000

The most occupied neutral and ionized side chain position in the full MCCE analysis is used for Asp 85 and 212. The energy of each microstate is then obtained from three terms: (1) the reaction field energy and electrostatic and Lennard-Jones interactions with the backbone and side chains with no conformational degrees of freedom for the SB and Asp 85 and 212 (A); (2) a mean field approximation of the contribution of the non-cluster side chains at pH 7 calculated using the MCCE position and ionization state conformer occupancies for all other residues (Eqn. 3, A); and (3) the appropriate pair-wise interactions amongst the 3 residues in each of the eight microstates (Eqn. 5, B).

Solution pK_a: pK_a of this residue type in isolation in solution. Contributions of the rest of the protein to the ionization of the cluster residues. Reaction field energy loss: the difference in the interactions with continuum water for the residue at its location in the protein and isolated in solution. Backbone interaction: sum of electrostatic and non-electrostatic interactions with backbone amides. These two energy terms are the same in full Monte Carlo sampling and in the reduced model. MFE': mean-field interaction energy of the rest of the protein conformers, is $\sum_k \Delta G_{ik}$ where Δ_k is the occupancy of the kth conformer in full Monte Carlo sampling and ΔG_{jk} the interaction energy with cluster conformer j. Δ_k can be pH dependent. The contribution of each residue to MFE' for calculations with explicit water are found in Appendix II. pK': pH when a group is 50% ionized inside the protein if there are no intra-cluster interactions (Eqn. 4). All energies are the difference in free energy of ionized and neutral conformer (Eqn. 1). All energies in ΔpK_a units. 1 ΔpK_a unit = 1.36 kcal/mol.

The central cluster, composed of 3 residues has eight microstates with different charge distributions. Microstate energy in ΔpK_a units (C) derived with equation 5 using the data in (A, B). Microstate occupancies are the Boltzmann distribution given these microstate energies. Significant values of the fractional occupancy are in bold. All energies in ΔpK_a units. 1 pK_a unit = 1.36 kcal/mol.

APPENDIX IV. Terms contributing to cluster microstate energies in the reduced model of the proton release cluster.

A. Interactions with the rest of the protein and the surrounding continuum water.

	Waters in cavities	Residue	Solution pK_a	Reaction field energy loss	Backbone Interaction	MFE'	pK'
bR	continuum	Glu194	4.75	8.74	-3.23	-8.8	1.46
		Glu204	4.75	8.68	-5.54	-9.4	-1.51
	explicit	Glu194	4.75	9.39	-3.25	-10.4	0.49
		Glu204	4.75	9.19	-5.3	-10.4	-1.76
early M	continuum	Glu194	4.75	9.49	-2.54	-12.7	-0.99
		Glu204	4.75	8.73	-5.64	-10.9	-3.07
	explicit	Glu194	4.75	9.78	-2.8	-13.1	-1.37
		Glu204	4.75	9.12	-5.79	-11.6	-3.53
late M	continuum	Glu194	4.75	7.99	-1.81	-10.5	0.42
		Glu204	4.75	7.81	-5.81	-8.9	-2.14
	explicit	Glu194	4.75	9.28	-2.4	-17.3	-5.66
		Glu204	4.75	8.87	-5.23	-10.6	-2.2

B. Pair-wise interactions between ionized and neutral conformers of the residues in the proton release cluster.

	Waters in cavities		$Glu204^0$	$Glu204^-$
bR	continuum	$Glu194^0$	1.32	-2.41
		$Glu194^-$	-1.51	14.00
	explicit	$Glu194^0$	0.52	-2.32
		$Glu194^-$	1.34	13.98
early M	continuum	$Glu194^0$	1.84	-3.19
		$Glu194^-$	-2.11	9.96
	explicit	$Glu194^0$	1.81	-3.28
		$Glu194^-$	-2.11	10.21
late M	continuum	$Glu194^0$	-0.37	-0.64
		$Glu194^-$	0.56	7.20
	explicit	$Glu194^0$	0.55	-1.73
		$Glu194^-$	-0.22	8.19

C. Energies and occupancies of the 4 microstates of the central cluster in the reduced model.

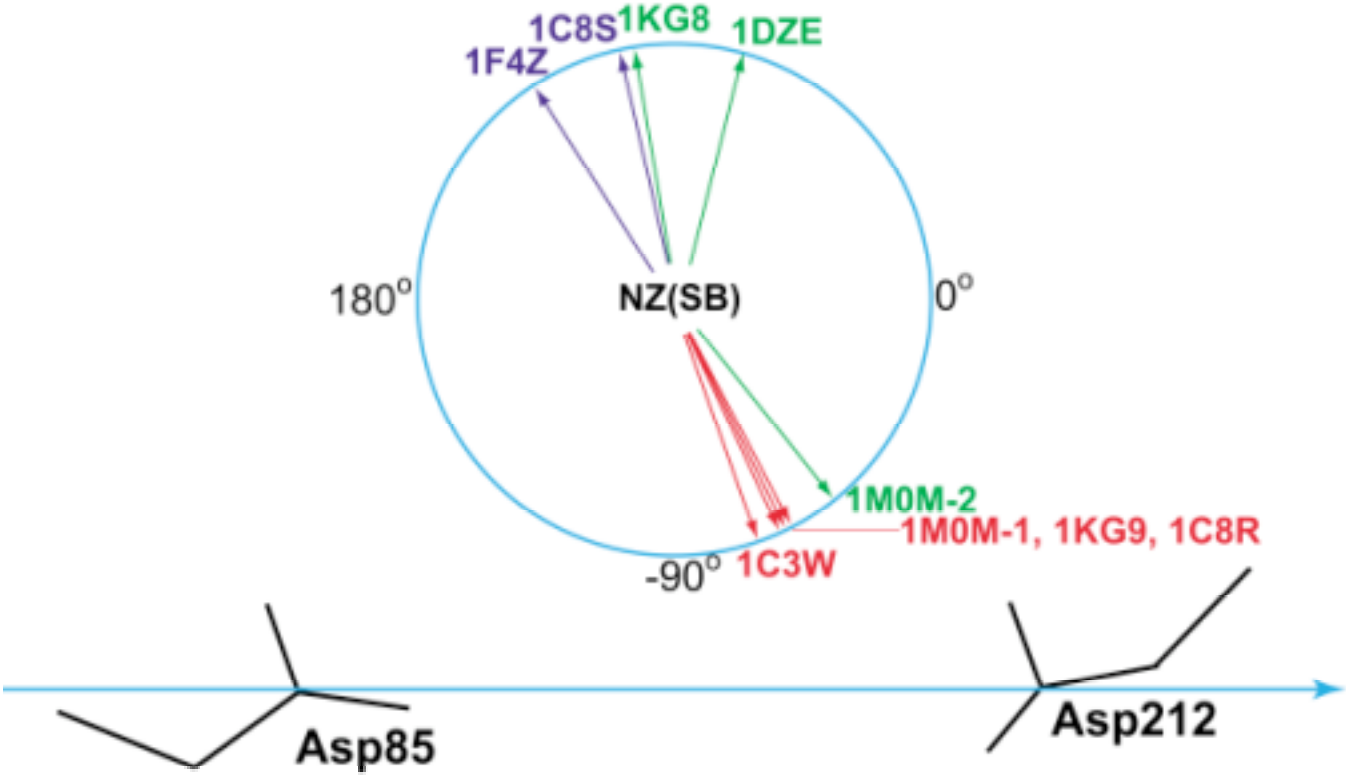
Continuum Water in Internal Cavities								
Microstates		Net charge	BR		Early M		Late M	
			Energy	Occupancy	Energy	Occupancy	Energy	Occupancy
$E194^0$	$E204^0$	0	1.3	0.000	1.8	0.000	-0.4	0.000
	$E204^-$	–1	-10.9	**1.000**	-13.3	**0.999**	-9.8	**0.949**
$E194^-$	$E204^0$	–1	-7.0	0.000	-10.1	0.001	-6.0	0.000
	$E204^-$	–2	0.0	0.000	-8.1	0.000	-8.5	0.051

Explicit Water in Internal Cavities								
$E194^0$	$E204^0$	0	0.5	0.000	1.8	0.000	0.5	0.000
	$E204^-$	–1	-16.9	**1.000**	-13.8	**1.000**	-11.0	0.002
$E194^-$	$E204^0$	–1	-12.8	0.000	-10.5	0.000	-12.9	**0.138**
	$E204^-$	–2	-7.2	0.000	-8.7	0.000	-13.7	**0.860**

All energy terms as described in Appendix III.

Appendix V. Position of the Schiff base relative to Asp85 and 212

A. Schiff Base orientation.



An angle, Γ, is defined for the SB C15-NZ-CE dipole using the CGs of Asp85 (C_G^{D85}) and Asp 212 (C_G^{D212}), the NZ of the Schiff base (N_Z^{SB}), and the midpoint of the SB C15 and CE (C_{mid}^{SB}). Asp85 and 212 CG positions change little between structures. Γ is the angle between $\overrightarrow{C_G^{D85}C_G^{D212}}$ and the projection of vector $\overrightarrow{C_{mid}^{SB}N_Z^{SB}}$ onto the plane of C_G^{D85}, C_G^{D212} and N_Z^{SB}. When the Schiff base is pointing down towards the two acids, Γ is negative. A value between –90 and 90 indicates the N is pointing towards Asp 212 rather than Asp 85 (Appendix I).

B. Distance between the Schiff base nitrogen and Asp85 and 212

PDB	1C8R	1C3W	1KG9	1M0M-1	1DZE	1KG8	1M0M-2	1C8S	1F4Z
Average Distance (Å)	4.34	4.29	4.36	4.27	4.97	5.15	4.30	4.91	4.84
Difference (Å)	-0.15	-0.33	-0.26	-0.54	-0.53	-0.34	0.13	0.27	-0.45

Average distance: between NZ of the Schiff base and CGs of Asp85 and 212. Difference: (SB_{NZ} to 212_{CG} distance) - (SB_{NZ} to 85_{CG} distance).