

SUPPLEMENTARY MATERIAL

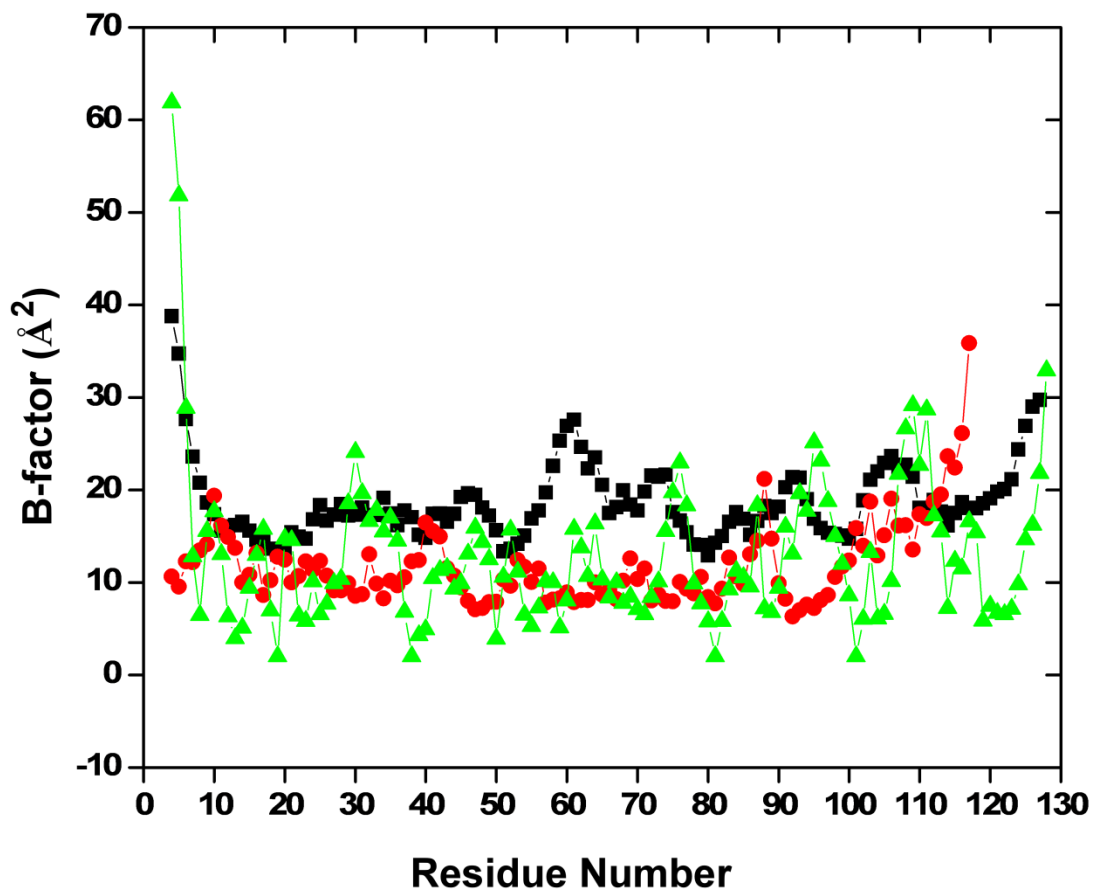
Crystal Structure of the *Aeropyrum pernix* L7Ae Multifunctional Protein and Insight into its Extreme Thermostability

Mohammad Wadud Bhuiya,* Jimmy Suryadi, Zhaoli Zhou, and Bernard A. Brown II*

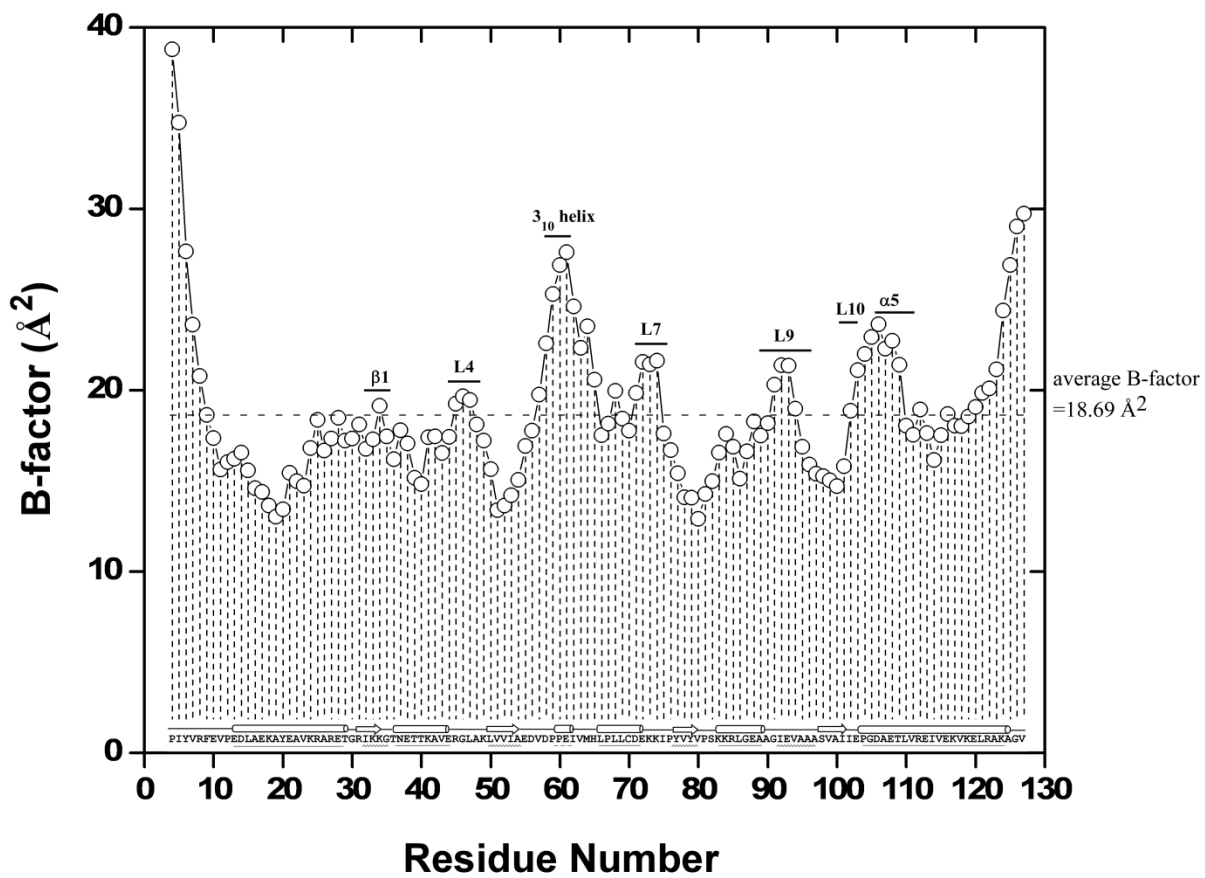
These studies were initiated in the Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, and Center for Structural Biology, Wake Forest University School of Medicine, Winston-Salem, NC 27157.

* Corresponding authors. Email: bebrown@wcsr.com, wadud@conagen-inc.com

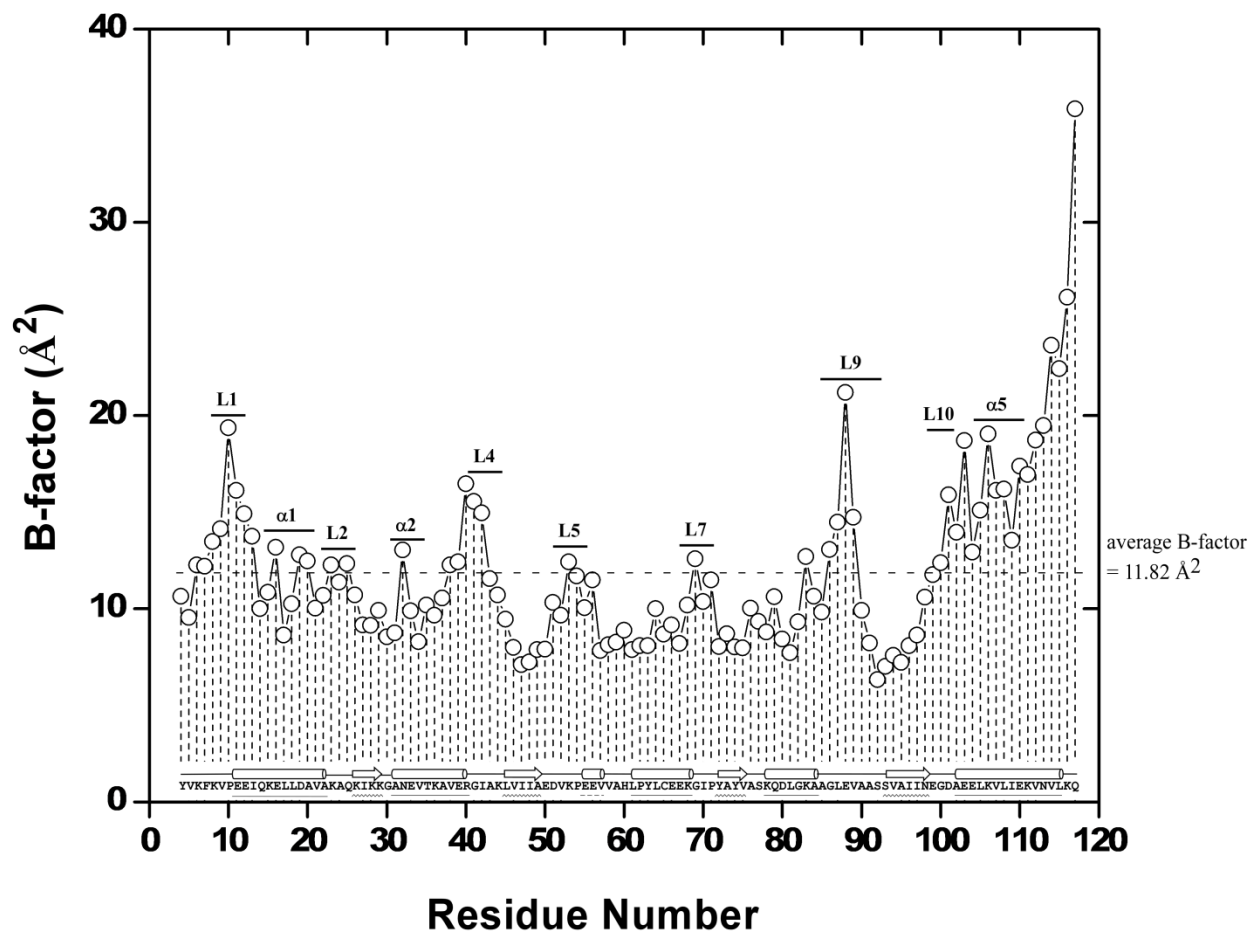
Present addresses: M. Wadud Bhuiya, Conagen Inc., Saint Louis, MO, 63132; USA; J. Suryadi, Department of Chemistry, Wake Forest University, Winston-Salem, NC, 27109; Z. Zhou, Bristol-Myers Squibb, Syracuse, NY, 13221; B. A. Brown II, Womble Carlyle Sandridge and Rice, LLP, One West Fourth Street, Winston-Salem, NC, 27695, USA.



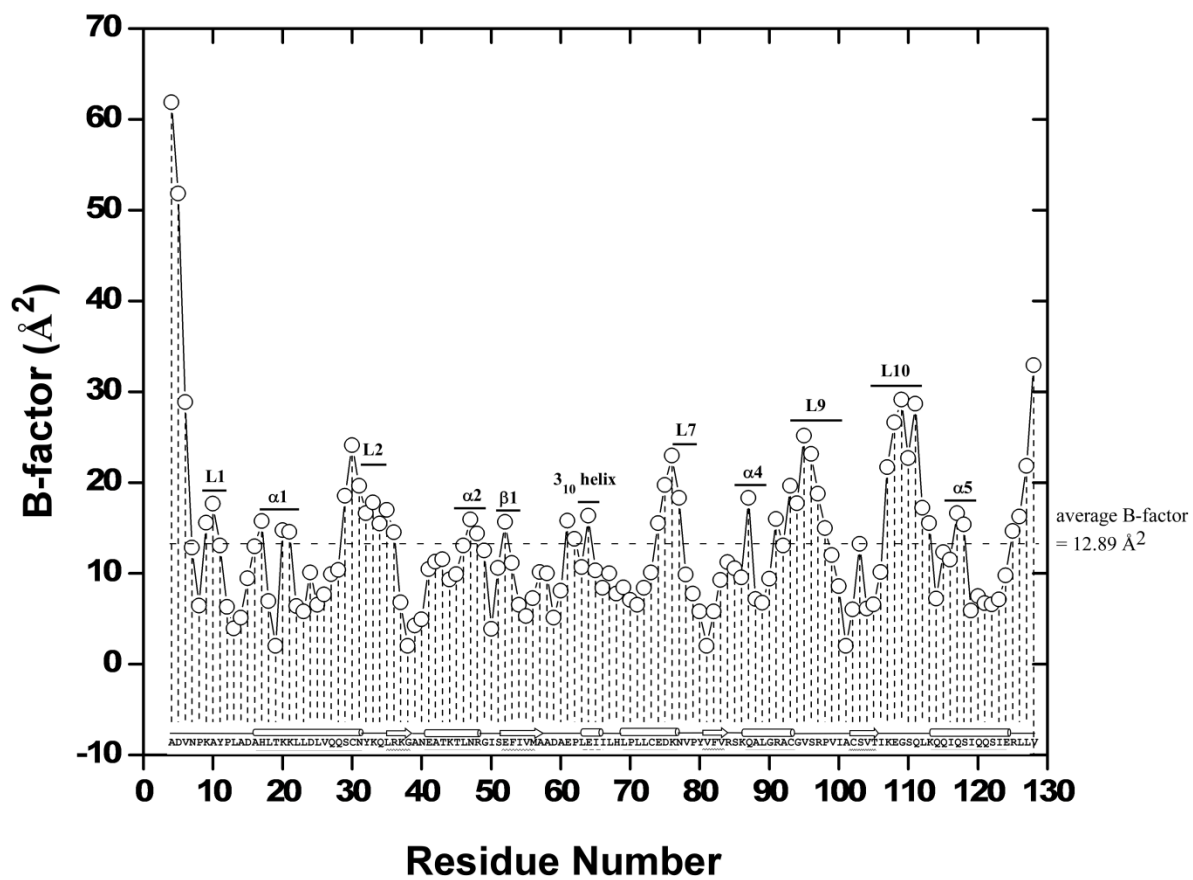
SUPPLEMENTARY FIGURE S1(a): The B-factors of main-chain atoms in Ap L7Ae (black), Mj L7Ae (red), and human 15.5kD (green) are plotted versus the residue number.



SUPPLEMENTARY FIGURE S1(b): The B-factors for the main-chain atoms of Ap L7Ae are plotted versus the residue number.



SUPPLEMENTARY FIGURE S1(c): The B-factors for the main-chain atoms of Mj L7Ae are plotted versus the residue number.



SUPPLEMENTARY FIGURE S1(d): The B-factors for the main-chain atoms of human 15.5kD are plotted versus the residue number.

Supplementary Figure S2: Sequence Alignment of 90 L7Ae Homologs

CLUSTAL W (1.83) multiple sequence alignment (90 Sequences)

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2129247 -----MKDMAVYVVKFKVPEEIQKELLDAVAQAQ---KIKKGANEVTKAVERGIAKLVI IAEDVQPEEIVAHLPPLCEEKIPYAYVASKQDLGKAAGLEVAASSVAIINEGDAEELK-VLIEKVNVLKQ----- 120
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3914933 -----MYVRFVPEDMQNEALSLEKVRRESGKIKKGTNETTKAVERGLAKLVVIAEDVDPEEIVAHLPPLCEEKIPYVYVPSKNDLGRVAVGIEVPCASAAIINEGELRKELGSLVEKIKGLQK----- 119
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91772287 -----MVKFEVPEDELADKALEALELARDTGKIKKGTNEATKAVERGVAQLVLI AEDIEPAEIVAHLPPLCEEKIPYVYVPSKNDLGRVAVGIEVPCASAAIINEGELRKELGSLVEKIKGLQK----- 117
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10962652 -----MPVYVDFVPELADKALEALELARDTGKIKKGTNEATKAVERGVAKLVI AEDVPEEIVMHLPELAEKGIIEVVFVETQDELGNAAGLEVGSAAAVVAAGDAEDEIEDISTKVEDLQ----- 120
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14324662 -----MEKSYVKFETPEDVSQKALDLVSAFSGKIKKGTNEVKSIERGESKLVVIAEDVNPPEVYVYVPSKNDLGRVAVGIEVPCASAAIINEGELRKELGSLVEKIKGLQK----- 121
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48431000 -----MES--YVKFQTPETLEKAVLDMVENSYKTKGKRVKGTNEVKSIERGESKLVVIAEDVNPPEVYVYVPSKNDLGRVAVGIEVPCASAAIINEGELRKELGSLVEKIKGLQK----- 127
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8923444 MTKIKADPDGPEAQAE~

A. pernix L7Ae

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          10      20      30      40      50      60      70      80      90      100     110     120     127
123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567
14601646 -----MSKPIYVRFVPEDLAEKAYEAVKRARETGRIRKGTNETTKAVERGLAKLVVIAEDVDPEEIVMHLPLLCDEKKIPYVYVPSKKRLGEAAGLEVAAASVAIIEPGDAETLVREIVEKVKELRAKAGV----- 127
          : * . : * : . : : * : : . . : : :
          I G N E K R L I D P V L C E V K L I

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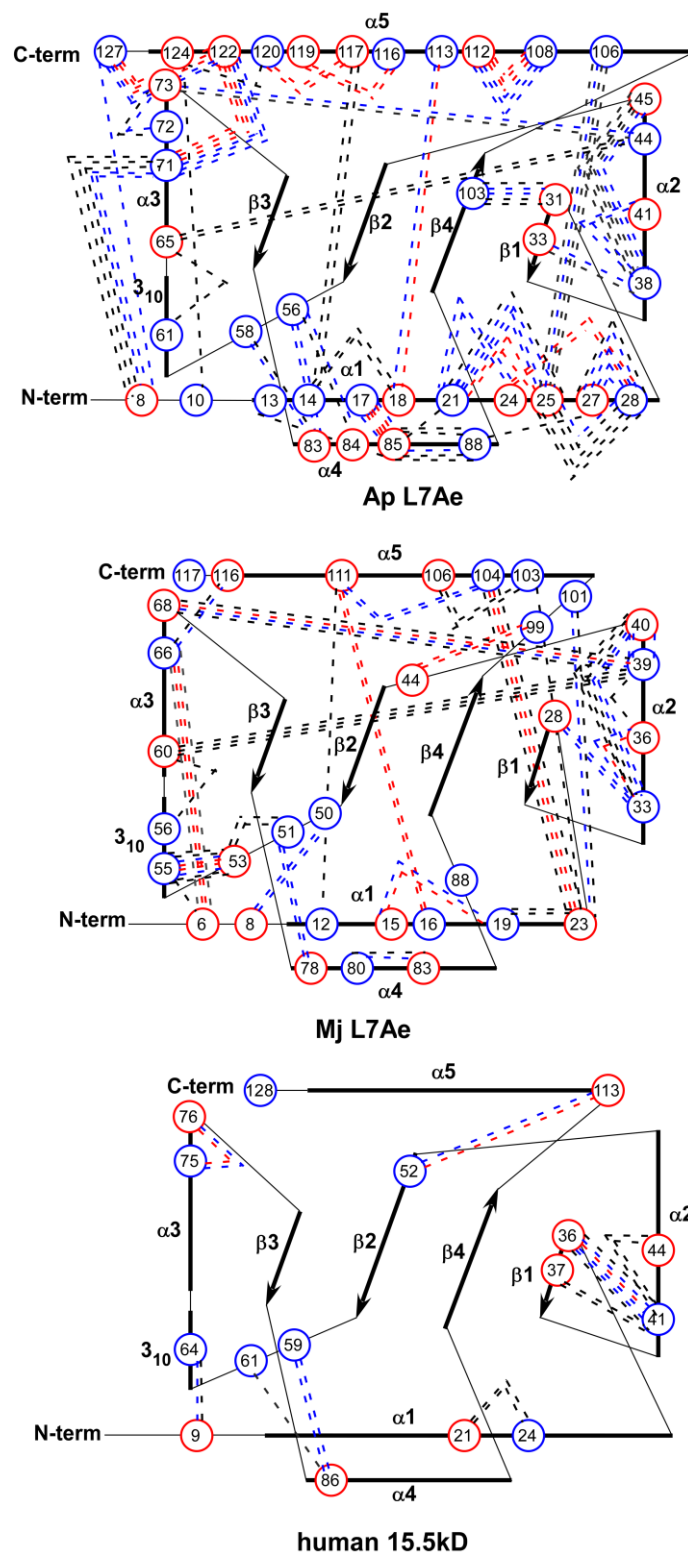
- * (3) G35, K41, P59 (N37)
- : (10) I32, E38, R45, I53, D56, I62, L66, K83, L86, I101
- . (5) N37, L50, C70, E72, Y79

Nonpolar AA:	A G I L M P V	Dark Gray
Uncharged polar AA	C N Q S T	Teal
Basic AA	H K R	Red
Acidic AA	D E	Blue
Aromatic AA	F Y W	Green

SUPPLEMENTARY FIGURE S2: ClustalW alignment of proteins with sequence similarity to *A. pernix* L7Ae (highlighted yellow). Sequence conservation is indicated by: “*”, identical residues; “:”, functional conservation; “.”, semi-conservation.

Uncharged polar AA	C N Q S T	Teal
Basic AA	H K R	Red
Acidic AA	D E	Blue
Aromatic AA	F Y W	Green

SUPPLEMENTARY FIGURE S3: Analysis of the secondary structure and functionality of *A. pernix* L7Ae amino acid residues. Amino acids are colored according to their characteristics as described in the figure. Conservation of amino acids by ConSurf and ClustalW alignments are indicated.^{81,83} Residues in α -helices are represented by straight lines; 3_{10} helices are indicated by dashed lines; and β -sheets are specified by saw-tooth lines. Loops, turns, and coils are indicated by blank spaces. ClustalW sequence alignment conservation is indicated by: “*”, identical residues; “.”, strong conservation; and “.”, semi-conservation. The residues contributing stability of the protein has been ranked based on ion pair formation of inter-(ER) and intra-(RA) secondary structure regions, and ion pair distance of two oppositely charged groups within 4, 6, and 8 Å, respectively.



SUPPLEMENTARY FIGURE S4: Schematic view of total ion pairs. The wide lines, arrows, and thin lines represent α -helices, β -strands, and loops, respectively. The red and blue circles enclosing a numeral are positive or negative charged residues at the indicated position. The red, blue, and black dotted lines represent ion pair networks within distances of 4, 6, and 8 Å, respectively.

SUPPLEMENTARY TABLE S1: Ion pairs in *A. pernix* L7Ae.

Ion pairs were scored based on the number of inter-(ER) and intra-(RA) secondary structure interactions and the distance between the two oppositely charged moieties. Interaction distances were converted to proportional values by taking the inverse of the distance times 10. Each interaction was characterized as “ER” or “RA”. Intersecondary structure (ER) interactions were awarded 2 points, and intrasecondary (RA) structure interactions were awarded 1 point. The total score for each ion pair interaction was the sum of the distance score and the ER/RA score. The total score for each amino acid residue was the sum of the scores for all interactions that involved that residue.

ATOM 1	ATOM 2	DISTANCE	DISTANCE SCORE	ER/RA	ER/RA SCORE	TOTAL
ARG8 NE	ASP71 OD1	6.48	1.5	ER		
	ASP71 OD2	4.61	2.2	ER		
	VAL127 OXT C-ter	5.06	2.0	ER		
ARG8 NH1	ASP71 OD2	6.12	1.6	ER		
ARG8 NH2	ASP71 OD1	7.23	1.4	ER		
	ASP71 OD2	5.14	1.9	ER		
ARG8 Total			10.7	6 ER	12	22.7
GLU10 OE2	LYS124 NZ	7.86	1.3	ER	2	3.3
GLU13 OE1	ARG85 NH1	6.58	1.5	ER	2	3.5
ASP14 OD1	LYS18 NZ	6.95	1.4	RA		
	LYS117 NZ	7.51	1.3	ER		
ASP14 OD2	LYS18 NZ	7.55	1.3	RA		
	LYS117 NZ	7.07	1.4	ER		
ASP14 Total			5.5	2 ER, 2 RA	6	11.5
GLU17 OE1	ARG85 NE	5.05	2.0	ER		
	ARG85 NH1	3.71	2.7	ER		
	ARG85 NH2	2.92	3.4	ER		
GLU17 OE2	ARG85 NE	4.92	2.0	ER		
	ARG85 NH1	2.84	3.5	ER		
	ARG85 NH2	3.49	2.9	ER		
GLU17 Total			16.5	6 ER	12	28.5
LYS18 NZ	ASP14 OD2	7.55	1.3	RA		
	GLU113 OE1	2.82	3.5	ER		
	GLU113 OE2	4.36	2.3	ER		
	ASP14 OD1	6.95	1.4	RA		
LYS18 Total			8.6	2 ER, 2 RA	6	14.6
GLU21 OE1	ARG25 NE	4.84	2.1	RA		
	ARG25 NH1	6.6	1.5	RA		
	ARG25 NH2	5.24	1.9	RA		
	ARG85 NH2	7.47	1.3	ER		
	LYS24 NZ	5.05	2.0	RA		
GLU21 OE1	ARG25 NE	2.75	3.6	RA		
	ARG25 NH1	4.41	2.3	RA		
	ARG25 NH2	3.23	3.1	RA		
	LYS24 NZ	4.77	2.1	RA		
GLU21 Total			19.9	1 ER, 8 RA	10	29.9
LYS24 NZ	GLU21 OE1	5.05	2.0	RA		
	GLU28 OE1	2.81	3.6	RA		
	GLU28 OE2	3.33	3.0	RA		
	GLU21 OE2	4.77	2.1	RA		
LYS24 Total			10.6	4 RA	4	14.6
ARG25 NE	GLU21 OE1	4.84	2.1	RA		

	GLU28 OE1	7.89	1.3	RA		
	GLU28 OE2	6.28	1.6	RA		
	ASP106 OD2	6.74	1.5	ER		
	GLU21 OE2	2.75	3.6	RA		
ARG25 NH1	GLU21 OE1	6.6	1.5	RA		
	GLU28 OE1	7.89	1.3	RA		
	GLU28 OE2	6	1.7	RA		
	ASP106 OD1	6.71	1.5	ER		
	ASP106 OD2	5.14	1.9	ER		
	GLU21 OE2	4.41	2.3	RA		
ARG25 NH2	GLU21 OE1	5.24	1.9	RA		
	GLU28 OE2	7	1.4	RA		
	ASP106 OD2	7.45	1.3	ER		
	GLU21 OE2	3.23	3.1	RA		
ARG25 Total			28.0	4 ER, 11 RA	19	47
ARG27 NE	GLU28 OE1	5.09	2.0	RA		
	GLU28 OE2	6.18	1.6	RA		
ARG27 NH1	GLU28 OE1	4.14	2.4	RA		
	GLU28 OE2	5.77	1.7	RA		
ARG27 NH2	GLU88 OE2	7.81	1.3	ER		
	GLU28 OE1	4.23	2.4	RA		
	GLU28 OE2	5.88	1.7	RA		
ARG27 Total			13.1	1 ER, 6 RA	8	31.1
GLU28 OE1	ARG25 NE	7.89	1.3	RA		
	ARG27 NE	5.09	2.0	RA		
	ARG25 NH1	7.89	1.3	RA		
	ARG27 NH1	4.14	2.4	RA		
	ARG27 NH2	4.23	2.4	RA		
	LYS24 NZ	2.81	3.6	RA		
GLU28 OE2	ARG25 NE	6.28	1.6	RA		
	ARG27 NE	6.18	1.6	RA		
	ARG25 NH1	6	1.7	RA		
	ARG27 NH1	5.77	1.7	RA		
	ARG25 NH2	7	1.4	RA		
	ARG27 NH2	5.88	1.7	RA		
	LYS24 NZ	3.33	3.0	RA		
GLU28 Total			25.6	13 RA	13	38.6
ARG31 NE	GLU103 OE1	6.71	1.5	ER		
	GLU103 OE2	4.7	2.1	ER		
ARG31 NH1	GLU103 OE2	6.57	1.5	ER		
ARG31 NH2	GLU103 OE1	6.99	1.4	ER		
	GLU103 OE2	4.79	2.1	ER		
ARG31 Total			8.7	5 ER	10	18.7
LYS33 NZ	GLU38 OE1	4.9	2.0	ER		
	GLU38 OE2	6.38	1.6	ER		
LYS33 Total			3.6	2 ER	4	7.6
GLU38 OE1	ARG45 NE	6.5	1.5	RA		
	ARG45 NH1	5.64	1.8	RA		
	ARG45 NH2	4.65	2.2	RA		
	LYS33 NZ	4.9	2.0	ER		
	LYS41 NZ	5.04	2.0	RA		
GLU38 OE2	ARG45 NE	7.95	1.3	RA		
	ARG45 NH1	6.56	1.5	RA		
	ARG45 NH2	6.15	1.6	RA		
	LYS33 NZ	6.38	1.6	ER		
	LYS41 NZ	4.38	2.3	RA		

GLU38 Total			17.7	2 ER, 8 RA	12	29.7
LYS41 NZ	GLU38 OE1	5.04	2.0	RA		
	GLU38 OE2	4.38	2.3	RA		
LYS41 Total			4.3	2 RA	2	6.3
GLU44 OE1	LYS73 NZ	4.43	2.3	ER		
GLU44 OE2	HIS65 NE2	7.72	1.3	ER		
	LYS73 NZ	6.54	1.5	ER		
GLU44 Total			5.1	3 ER	6	11.1
ARG45 NE	GLU38 OE1	6.5	1.5	ER		
	GLU38 OE2	7.95	1.3	ER		
ARG45 NH1	GLU38 OE1	5.64	1.8	ER		
	GLU38 OE2	6.56	1.5	ER		
ARG45 NH1	GLU38 OE1	4.65	2.2	ER		
	GLU38 OE2	6.15	1.6	ER		
ARG45 Total			9.9	6 ER	12	21.9
ASP56 OD1	LYS83 NZ	4.97	2.0	ER		
	LYS84 NZ	6.25	1.6	ER		
ASP56 OD2	LYS83 NZ	5.04	2.0	ER		
	LYS84 NZ	4.52	2.2	ER		
ASP56 Total			7.8	4 ER	8	15.8
ASP58 OD1	LYS83 NZ	6.77	1.5	ER		
ASP58 OD2	LYS83 NZ	6.02	1.7	ER		
ASP58 Total			3.1	2 ER	4	7.1
GLU61 OE2	HIS65 ND1	6.18	1.6	RA	1	2.6
HIS65 ND1	GLU61 OE2	6.18	1.6	RA		
HIS65 NE2	GLU44 OE2	7.72	1.3	ER		
HIS65 Total			2.9	1 ER, 1 RA	3	5.9
ASP71 OD1	ARG8 NE	6.48	1.5	ER		
	ARG122 NE	4.78	2.1	ER		
	ARG122 NH1	2.98	3.4	ER		
	ARG8 NH2	7.23	1.4	ER		
	ARG122 NH2	3.02	3.3	ER		
ASP71 OD2	ARG8 NE	4.61	2.2	ER		
	ARG122 NE	5.3	1.9	ER		
	ARG8 NH1	6.12	1.6	ER		
	ARG122 NH1	4.23	2.4	ER		
	ARG8 NH2	5.14	1.9	ER		
	ARG122 NH2	3.23	3.1	ER		
ASP71 Total			24.8	11 ER	22	42.8
GLU72 OE1	LYS73 NZ	5.38	1.9	RA		
GLU72 OE2	LYS73 NZ	7.33	1.4	RA		
GLU72 Total			3.2	2 RA	2	5.2
LYS73 NZ	GLU72 OE2	7.33	1.4	RA		
	GLU72 OE1	5.38	1.9	RA		
	GLU44 OE1	4.43	2.3	ER		
	GLU44 OE2	6.54	1.5	ER		
LYS73 Total			7.0	2 ER, 2 RA	6	13
LYS83 NZ	ASP58 OD1	6.77	1.5	ER		
	ASP58 OD2	6	1.7	ER		
	ASP56 OD1	4.97	2.0	ER		
	ASP56 OD2	5.04	2.0	ER		
LYS83 Total			7.1	4 ER	8	15.1
LYS84 NZ	ASP56 OD1	6.25	1.6	ER		
	ASP56 OD2	4.52	2.2	ER		
LYS84 Total			3.8	2 ER	4	7.8

ARG85 NE	GLU17 OE2	4.92	2.0	ER		
	GLU88 OE1	4.94	2.0	ER		
	GLU88 OE2	6.82	1.5	RA		
	GLU17 OE1	5.05	2.0	ER		
ARG85 NH1	GLU17 OE2	2.84	3.5	ER		
	GLU13 OE1	6.58	1.5	ER		
	GLU88 OE1	7.22	1.4	RA		
	GLU17 OE1	3.71	2.7	ER		
ARG85 NH2	GLU17 OE2	3.49	2.9	ER		
	GLU88 OE1	6.19	1.6	RA		
	GLU88 OE2	7.69	1.3	RA		
	GLU17 OE1	2.92	3.4	ER		
	GLU21 OE1	7.47	1.3	ER		
ARG85 Total			27.2	9 ER, 4 RA	22	49.2
GLU88 OE1	ARG85 NE	4.94	2.0	RA		
	ARG85 NH1	7.22	1.4	RA		
	ARG85 NH2	6.19	1.6	RA		
GLU88 OE2	ARG85 NE	6.82	1.5	RA		
	ARG27 NH2	7.81	1.3	ER		
	ARG85 NH2	7.69	1.3	ER		
GLU88 Total			9.1	2 ER, 4 RA	8	17.1
GLU103 OE1	ARG31 NE	6.71	1.5	ER		
	ARG31 NH2	6.99	1.4	ER		
GLU103 OE2	ARG31 NE	4.7	2.1	ER		
	ARG31 NH1	6.57	1.5	ER		
	ARG31 NH2	4.79	2.1	ER		
GLU103 Total			8.7	5 ER	10	18.7
ASP106 OD1	ARG25 NH1	6.71	1.5	ER		
ASP106 OD2	ARG25 NE	6.74	1.5	ER		
	ARG25 NH1	5.14	1.9	ER		
	ARG25 NH2	7.45	1.3	ER		
ASP106 Total			6.3	4 ER	8	14.3
GLU108 OE1	ARG112 NE	2.99	3.3	RA		
	ARG112 NH1	2.91	3.4	RA		
	ARG112 NH2	3.56	2.8	RA		
GLU108 OE2	ARG112 NE	5.05	2.0	RA		
	ARG112 NH1	4.47	2.2	RA		
	ARG112 NH2	4.88	2.0	RA		
GLU108			15.9	6 RA	6	21.9
ARG112 NE	GLU108 OE1	2.99	3.3	RA		
	GLU108 OE2	5.05	2.0	RA		
ARG112 NH1	GLU108 OE1	2.91	3.4	RA		
	GLU108 OE2	4.47	2.2	RA		
ARG112 NH2	GLU108 OE1	3.56	2.8	RA		
	GLU108 OE2	4.88	2.0	RA		
ARG112 Total			15.9	6 RA	6	21.9
GLU113 OE1	LYS18 NZ	2.82	3.5	ER		
GLU113 OE2	LYS18 NZ	4.36	2.3	ER		
GLU113 Total			5.8	2 ER	4	9.8
GLU116 OE1	LYS119 NZ	2.74	3.6	RA		
GLU116 OE2	LYS119 NZ	3.28	3.0	RA		
GLU116 Total			6.7	2 RA	2	8.7
LYS117 NZ	GLU120 OE2	3.13	3.2	RA		
	GLU120 OE1	3	3.3	RA		
	ASP14 OD2	7.07	1.4	ER		
	ASP14 OD1	7.51	1.3	ER		

LYS117 Total			9.3	2 ER, 2 RA	6	15.3
LYS119 NZ	GLU116 OE2	3.28	3.0	RA		
	GLU116 OE1	2.74	3.6	RA		
LYS119 Total			6.7	2 RA	2	8.7
GLU120 OE1	LYS117 NZ	3	3.3	RA		
GLU120 OE2	LYS117 NZ	3.13	3.2	RA		
	LYS124 NZ	7.39	1.4	RA		
GLU120			7.9	3 RA	3	10.9
ARG122 NE	ASP71 OD1	4.78	2.1	ER		
	ASP71 OD2	5.3	1.9	ER		
	VAL127 O C-ter	2.95	3.4	RA		
ARG122 NH1	ASP71 OD1	2.98	3.4	ER		
	ASP71 OD2	4.23	2.4	ER		
	VAL127 O C-ter	5	2.0	RA		
	VAL127 OXT C-ter	4.75	2.1	RA		
ARG122 NH2	ASP71 OD1	3.02	3.3	ER		
	ASP71 OD2	3.23	3.1	ER		
	VAL127 O C-ter	3.61	2.8	RA		
	VAL127 OXT C-ter	2.73	3.7	RA		
ARG122 Total			30.0	6 ER, 5 RA	17	47
LYS124 NZ	GLU10 OE2	7.86	1.3	ER		
	GLU120 OE2	7.39	1.4	RA		
LYS124 Total			2.6	1 ER, 1 RA	3	5.6
VAL127 O C-ter	ARG122 NE	2.95	3.4	RA		
	ARG122 NH2	3.61	2.8	RA		
	ARG122 NH1	5	2.0	RA		
VAL127 OXT C-ter	ARG122 NH2	2.73	3.7	RA		
	ARG122 NH1	4.75	2.1	RA		
	ARG8 NE	5.06	2.0	RA		
VAL127 Total			15.9	6 RA	6	21.9

RESIDUE	TOTAL	DISTANCE SCORE	ER/RA SCORE
R8	22.7	10.7	12
E10	3.3	1.3	2
E13	3.5	1.5	2
D14	11.5	5.5	6
E17	28.5	16.5	12
K18	14.6	8.6	6
E21	29.9	19.9	10
K24	14.6	10.6	4
R25	47	28.0	19
R27	31.1	13.1	8
E28	38.6	25.6	13
R31	18.7	8.7	10
K33	7.6	3.6	4
E38	29.7	17.7	12
K41	6.3	4.3	2
E44	11.1	5.1	6
R45	21.9	9.9	12
D56	15.8	7.8	8
D58	7.1	3.1	4
E61	2.6	1.6	1

H65	5.9	2.9	3
D71	42.8	24.8	22
E72	5.2	3.2	2
K73	13	7.0	6
K83	15.1	7.1	8
K84	7.8	3.8	4
R85	49.2	27.2	22
E88	17.1	9.1	8
E103	18.7	8.7	10
D106	14.3	6.3	8
E108	21.9	15.9	6
R112	21.9	15.9	6
E113	9.8	5.8	4
E116	8.7	6.7	2
K117	15.3	9.3	6
K119	8.7	6.7	2
E120	10.9	7.9	3
R122	47	30.0	17
K124	5.6	2.6	3
V127	21.9	15.9	6

RESIDUE	TOTAL	DISTANCE SCORE	ER/RA SCORE	RANK
R85	49.2	27.2	22	1
R25	47	28.0	19	2
R122	47	30.0	17	3
D71	42.8	24.8	22	4
E28	38.6	25.6	13	5
R27	31.1	13.1	8	6
E21	29.9	19.9	10	7
E38	29.7	17.7	12	8
E17	28.5	16.5	12	9
R8	22.7	10.7	12	10
R45	21.9	9.9	12	11
V127	21.9	15.9	6	14
E108	21.9	15.9	6	12
R112	21.9	15.9	6	13
E103	18.7	8.7	10	16
R31	18.7	8.7	10	15
E88	17.1	9.1	8	17
D56	15.8	7.8	8	18
K117	15.3	9.3	6	19
K83	15.1	7.1	8	20
K18	14.6	8.6	6	21
K24	14.6	10.6	4	22
D106	14.3	6.3	8	23
K73	13	7.0	6	24
D14	11.5	5.5	6	25
E44	11.1	5.1	6	26
E120	10.9	7.9	3	27
E113	9.8	5.8	4	28
E116	8.7	6.7	2	29
K119	8.7	6.7	2	30
K84	7.8	3.8	4	31
K33	7.6	3.6	4	32
D58	7.1	3.1	4	33

K41	6.3	4.3	2	34
H65	5.9	2.9	3	35
K124	5.6	2.6	3	36
E72	5.2	3.2	2	37
E13	3.5	1.5	2	38
E10	3.3	1.3	2	39
E61	2.6	1.6	1	40