STRUCTURAL BASIS OF RECOGNITION OF PATHOGEN-ASSOCIATED MOLECULAR PATTERNS AND INHIBITION OF PRO-INFLAMMATORY CYTOKINES BY CAMEL PEPTIDOGLYCAN RECOGNITION PROTEIN

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Running title: Structural basis of recognition of PAMPs by PGRP-S

Keywords: PGRP-S, PAMPs, Crystal structure, Innate immunity, Peptidoglycan, Lipopolysaccharide, Lipoteichoic acid

Abbreviations: CPGRP-S, Camel peptidoglycan recognition protein-S; PMMPs, Pathogen-associated molecular patterns; LPS, Lipopolysaccharide; LTA, Lipoteichoic acid; PGN, Peptidoglycan.

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LEGENDS TO FIGURES

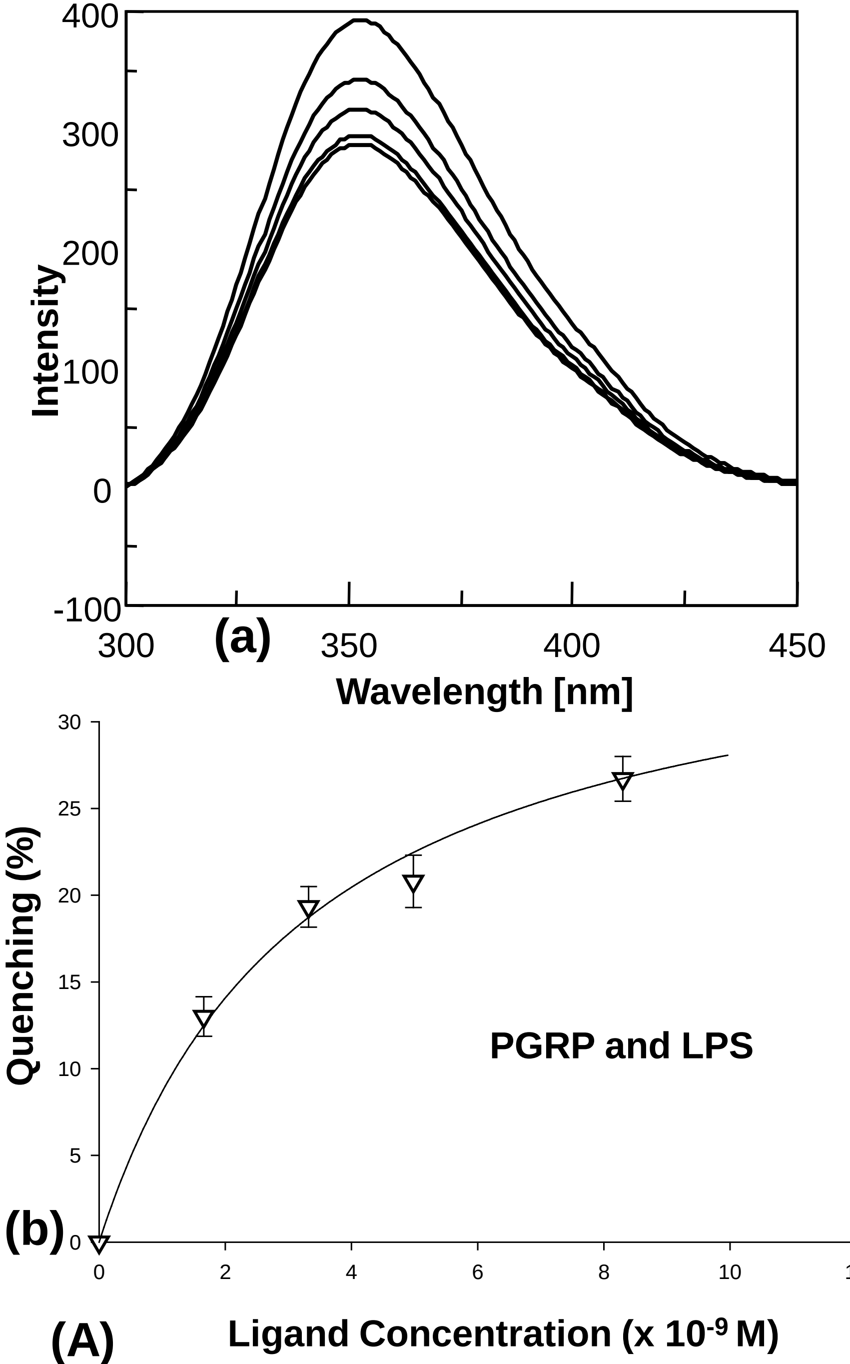
Figure S1. (a) Effect of the concentrations on the quenching of fluorescence. The fluorescence intensities are plotted for various concentrations of (A) LPS and (B) LTA. (b) The binding curves drawn for percentage quenching (Q = Fo-Fc/Fo)% against ligand/protein molar ratios for (A) LPS and (B) LTA.

Figure S2. The SPR-sensograms for the bindings of (A) LPS and (B) LTA with CPGRP-S. The protein was immobilized on the chip and the increasing concentrations (1.4 mM, 2.5 mM and 5.0 mM) of ligands LPS and LTA were used in mobile phase in separate experiments corresponding to curves a, b and c.

Figure S3. Effect of PGRP on the survival rate of mice after LPS-induced septic shock. Survival graph: 8 weeks old mice were injected intra-peritoneally with lethal dose of LPS (30 mg/kg) only (blue) and PGRP-LPS group were given LPS (30 mg/kg) prior to administering CPGRP (10 mg/kg) (red). The percentage of mice survival was analyzed, and the log-rank *t* test *P* values were calculated.

Figure S4. Sequence alignment of PGRP-S from various species performed with clustal W. The critical residues at the interfaces of molecules A and B (green), A and C (cyan) and C and D (red) are indicated. Cysteine residues shown in yellow colour. (The abbreviations: CPGRP-S for camel, BPGRP-S for bovine, HPGRP-S for human, MPGRP-S for mouse, RPGRP-S for rat and DPGRP-S for drosophila)

Figure S1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200
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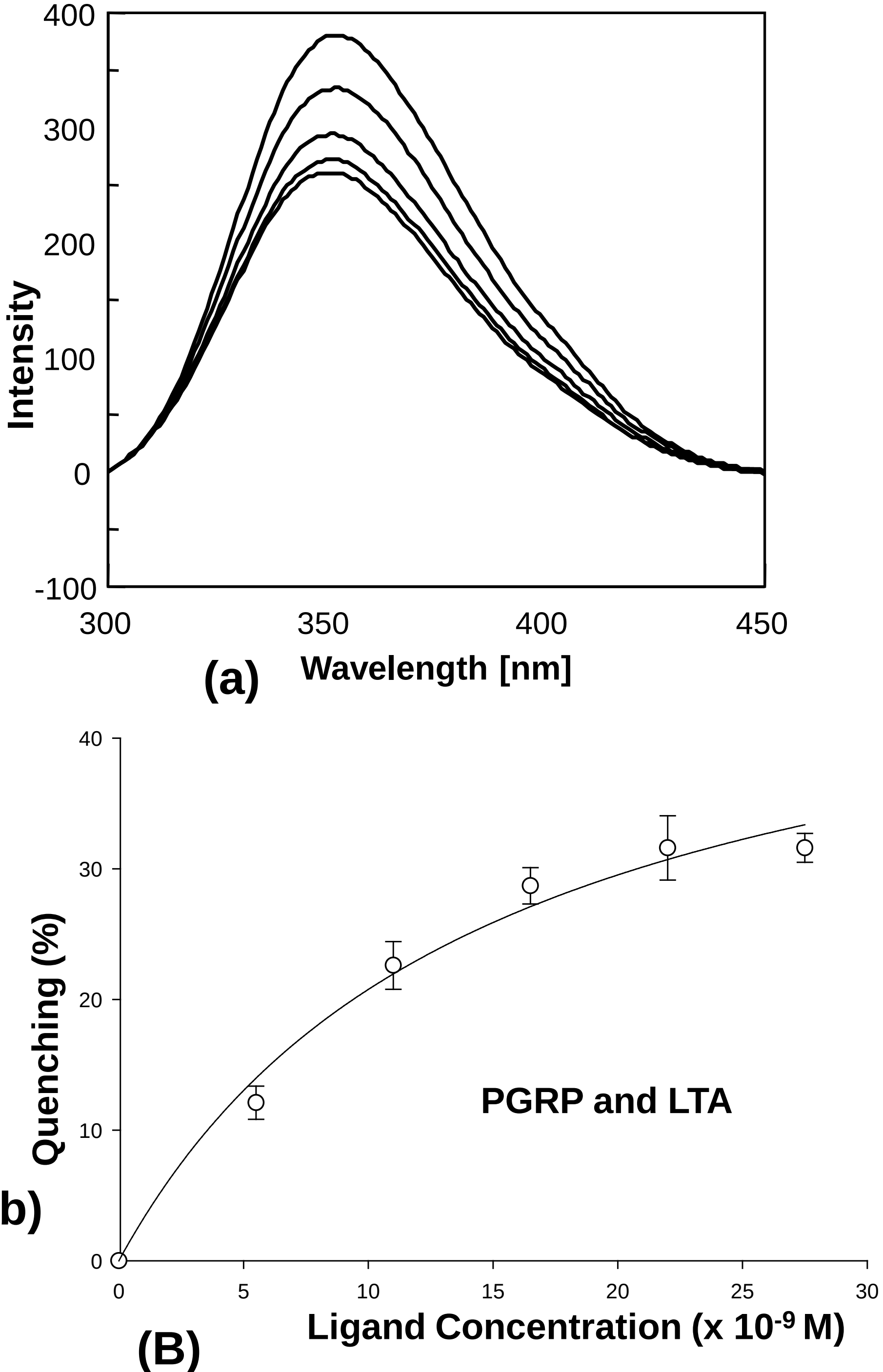
0
-100
Intensity
300
350
400
450
Wavelength [nm]
(a)
30
25
20
15
10
5
0
Quenching (%)
0
2
4
6
8
10
12
Ligand Concentration (x 10^{-9} M)
PGRP and LPS
(b)
(A)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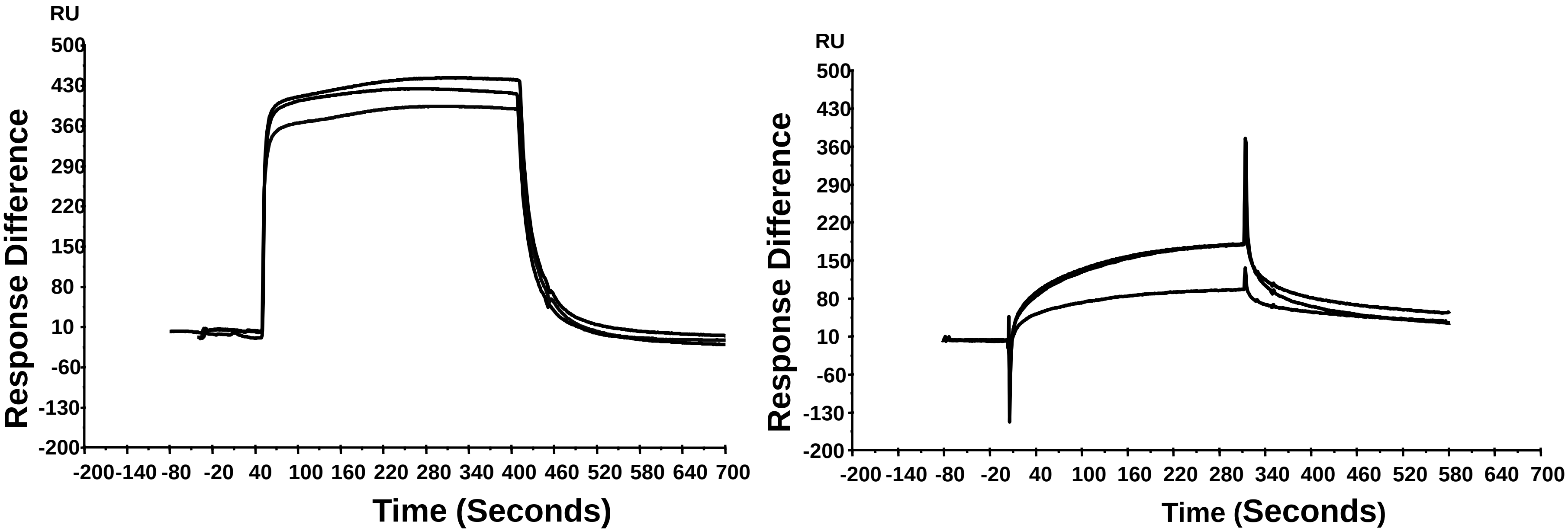
300
200
100
0
-100
Intensity
300
350
400
450
Wavelength [nm]
(a)
40
30
20
10
0
Quenching (%)
0
5
10
15
20
25
30
Ligand Concentration (x 10^{-9} M)
PGRP and LTA
(b)
(B)

Figure S2



LPS

LTA

(A)

(B)

Figure S3

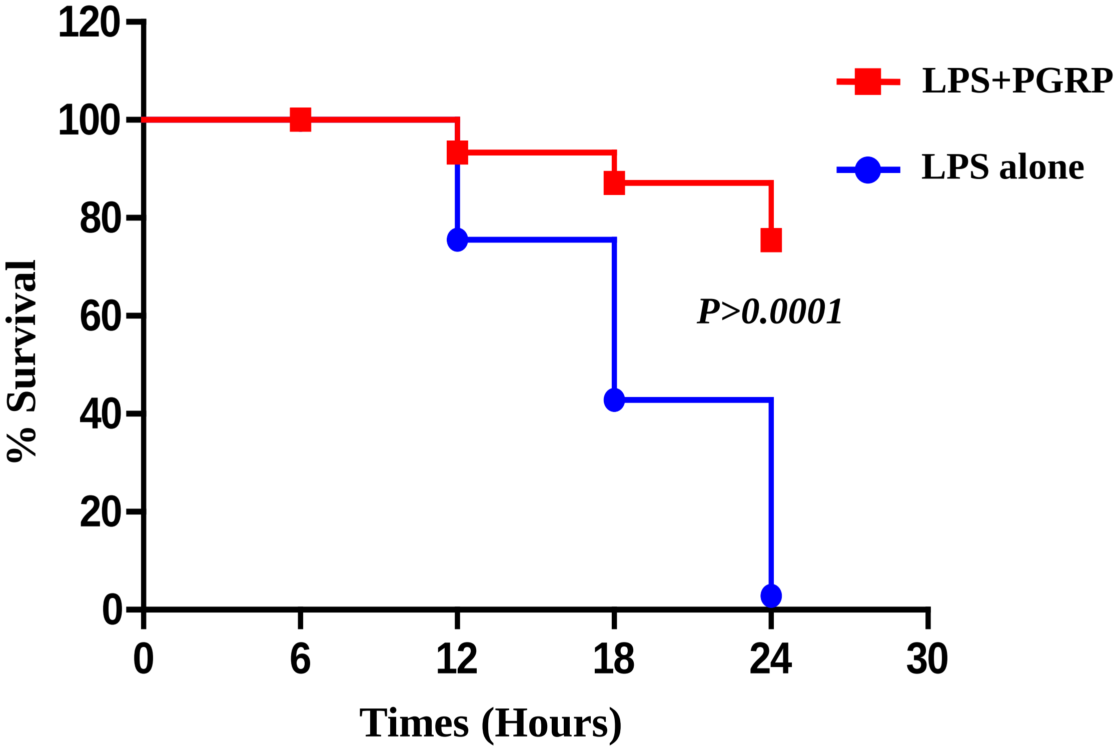
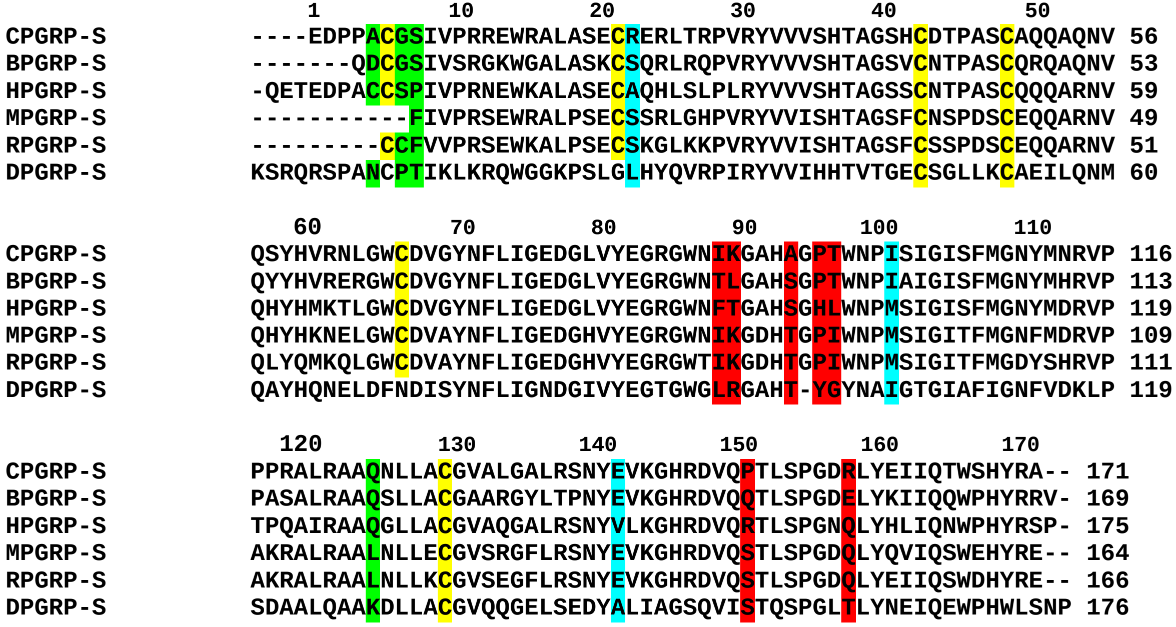

120
100
80
60
40
20
0
% Survival
0
6
12
18
24
30
Times (Hours)
LPS+PGRP
LPS alone
P>0.0001

Figure S4



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                     1        10        20        30        40         50
CPGRP-S          ----EDPPACGSIVPRREWRALASECRERLTRPVRYVVVSHTAGSHCDTPASCAQQAQNV 56
BPGRP-S          -------QDCGSIVSRGKWGALASKCSQRLRQPVRYVVVSHTAGSVCNTPASCQRQAQNV 53
HPGRP-S          -QETEDPACCSPIVPRNEWKALASECAQHLSLPLRYVVVSHTAGSSCNTPASCQQQARNV 59
MPGRP-S          -----------FIVPRSEWRALPSECSSRLGHPVRYVVISHTAGSFCNSPDSCEQQARNV 49
RPGRP-S          ---------CCFVVPRSEWKALPSECSKGLKKPVRYVVISHTAGSFCSSPDSCEQQARNV 51
DPGRP-S          KSRQRSPANCPTIKLKRQWGGKPSLGLHYQVRPIRYVVIHHTVTGECSGLLKCAEILQNM 60

                    60         70       80        90       100        110
CPGRP-S          QSYHVRNLGWCDVGYNFLIGEDGLVYEGRGWNIKGAHAGPTWNPISIGISFMGNYMNRVP 116
BPGRP-S          QYYHVRERGWCDVGYNFLIGEDGLVYEGRGWNTLGAHSGPTWNPIAIGISFMGNYMHRVP 113
HPGRP-S          QHYHMKTLGWCDVGYNFLIGEDGLVYEGRGWNFTGAHSGHLWNPMSIGISFMGNYMDRVP 119
MPGRP-S          QHYHKNELGWCDVAYNFLIGEDGHVYEGRGWNIKGDHTGPIWNPMSIGITFMGNFMDRVP 109
RPGRP-S          QLYQMKQLGWCDVAYNFLIGEDGHVYEGRGWTIKGDHTGPIWNPMSIGITFMGDYSHRVP 111
DPGRP-S          QAYHQNELDFNDISYNFLIGNDGIVYEGTGWGLRGAHT-YGYNAIGTGIAFIGNFVDKLP 119

                   120        130       140      150       160       170
CPGRP-S          PPRALRAAQNLLACGVALGALRSNYEVKGHRDVQPTLSPGDRLYEIIQTWSHYRA-- 171
BPGRP-S          PASALRAAQSLLACGAARGYLTPNYEVKGHRDVQQTLSPGDELYKIIQQWPHYRRV- 169
HPGRP-S          TPQAIRAAQGLLACGVAQGALRSNYVLKGHRDVQRTLSPGNQLYHLIQNWPHYRSP- 175
MPGRP-S          AKRALRAALNLLECGVSRGFLRSNYEVKGHRDVQSTLSPGDQLYQVIQSWEHYRE-- 164
RPGRP-S          AKRALRAALNLLKCGVSEGFLRSNYEVKGHRDVQSTLSPGDQLYEIIQSWDHYRE-- 166
DPGRP-S          SDAALQAAKDLLACGVQQGELSEDYALIAGSQVISTQSPGLTLYNEIQEWPHWLSNP 176
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🟩 A-B Interface

🟦 A-C Interface

🟥 C-D Interface

Table S1: Hydrogen bond distances (Å) between protein and LPS atoms. The alphabets in the parentheses indicate chain identifications.

Protein Atoms	Water Oxygen	LPS Atoms	Distance (Å)
(A) Arg 170 Nε		O6	3.3
	W207 (A)	O6	3.1
(A) Glu 142 Oε2	W207 (A)		2.8
	W209 (C)	O46	2.5
(C) Pro 96 O	W209 (C)		2.8
(C) Pro 96 O		O46	3.3
(C) Asn 99 Nδ2		O46	3.3
(C) Gly 95 O		O46	3.4
	W209 (C)	O47	3.2
	W296 (C)	O47	3.4
(C) Trp 66 Nε1		O4	3.0
	W179 (C)	O4	2.7
(C) Arg 85 NH2	W179 (C)		2.9
(C) Lys 90 Nζ		O48	2.8
	W260 (C)	O48	2.5
	W260 (C)	O5	2.5
(C) Lys 90 Nζ	W260 (C)		2.5
(C) Gly 91 O	W260 (C)		2.7
(C) Ala 92 O		O7	3.4
(C) His 93 N		O7	3.0
(C) Asn 99 Nδ2		O7	2.7
(C) Asn 99 Nδ2		O1	2.5
	W179 (D)	O42	3.2
(D) Thr 97 N	W179 (D)		2.8
(D) Thr 97 N		O42	3.2
(D) Thr 97 Oγ1		O42	2.5
(D) Val 149 O		O43	2.5
(D) Gln 150 Nε2		O43	2.5
	W179 (D)	O43	2.9
(D) Gln 150 Nε2		O3	3.4
	W325 (C)	N2	3.4
(D) Gln 150 Oε1	W325 (C)		2.9

Table S2: Hydrogen bond distances (Å) between protein and LTA atoms. The alphabets in the parentheses indicate chain identifications.

Protein Atoms	Water Oxygen	LPS Atoms	Distance (Å)
(A) Asn 140 Nδ2		O9	2.8
	W310 (A)	O13	3.3
(A) Ser 139 Oγ	W310 (A)		3.0
(C) Asn 99 Nδ2		O2	2.8
(C) Gly 95 N		O2	3.4
(C) Asn 99 Nδ2		O4	2.9
	W174 (C)	O4	2.7
	W224 (C)	O4	3.3
(C) Lys 90 Nζ	W224 (C)		2.7
(C) His 93 O		N42	3.2
	W322 (C)	O5	3.0
(C) Arg 85 NH2	W322 (C)		2.6
(C) His 93 N		O41	3.4
(C) Ala 92 O			3.2
(C) Gln150 Nε2		N44	3.1
(D) Gln150 Nε2		O48	2.6
(D) Gly 95 N		O48	2.9
	W471 (D)	O48	3.2
(D) His 93 N	W471 (D)		2.9
(D) Gln 150 Oε1		O3	2.7
(D) Thr 97 Oγ1		O10	2.8
	W433 (D)	O11	3.0
(D) Ala 94 O	W433 (D)		2.7
(D) Val 149 O		O11	3.2
(D) Trp 98 Nε1		O13	3.4
	W203 (C)	O42	3.2
(D) Gln150 Oε1		O42	3.4
(D) Gln150 Nε2		O42	2.6