MULTI-LIGAND SPECIFICITY OF PATHOGEN ASSOCIATED MOLECULAR PATTERNS-BINDING SITE IN PEPTIDOGLYCAN RECOGNITION PROTEIN

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Running title: Multi-ligand binding site in CPGRP-S

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Abbreviations: CPGRP-S, Camel peptidoglycan recognition protein-S; PAMPs, Pathogen-associated molecular patterns; GlcNAc, N-acetylglucosamine; PGN, Peptidoglycan; MDP, Muramyl dipeptide.

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LEGENDS TO FIGURES

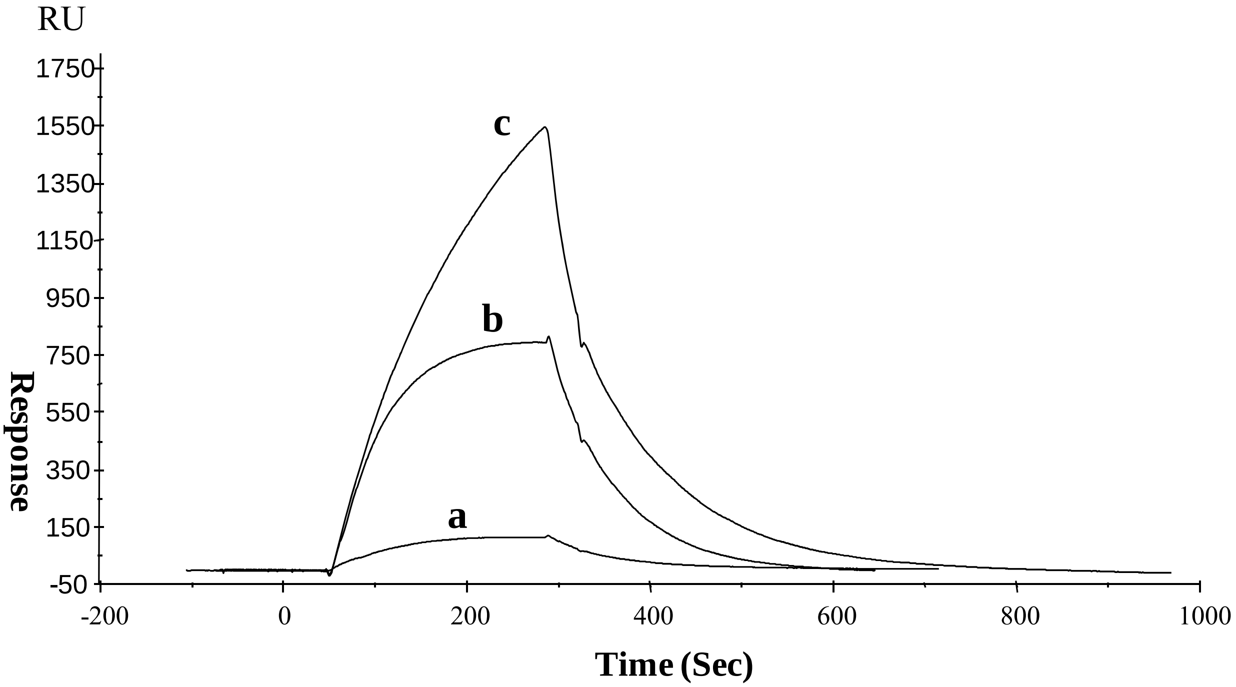
Figure S1. SPR sensograms for the bindings of (A) MDP and (B) GlcNAc and β-maltose with CPGRP-S. The protein was immobilized on CM-5 chip and increasing concentrations (1.4mM, 2.5mM and 5.0mM) of analytes MDP and GlcNAc/β-maltose were used in mobile phase in separate experiments corresponding to the curves a, b and c respectively.

Figure S2. Cartoon diagram showing the position of MDP (represented as the space filling model). The grid lines indicate the presence of intermolecular hydrogen bonded interactions in the tetramer.

Figure S3. Cartoon diagram showing the positions of GlcNAc and β-maltose in the binding cleft of the interface of molecules C and D. The grid lines indicate the presence of intermolecular hydrogen bonded interactions in the tetramer.

Figure S4. Alignment of the amino acid sequences of PGRP-S from various species. Cys residues are highlighted in yellow. The important residues responsible for the dimerization of molecules C and D and key to the formation of multi-ligand binding site are indicated in cyan.

Figure S1 (A)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Response
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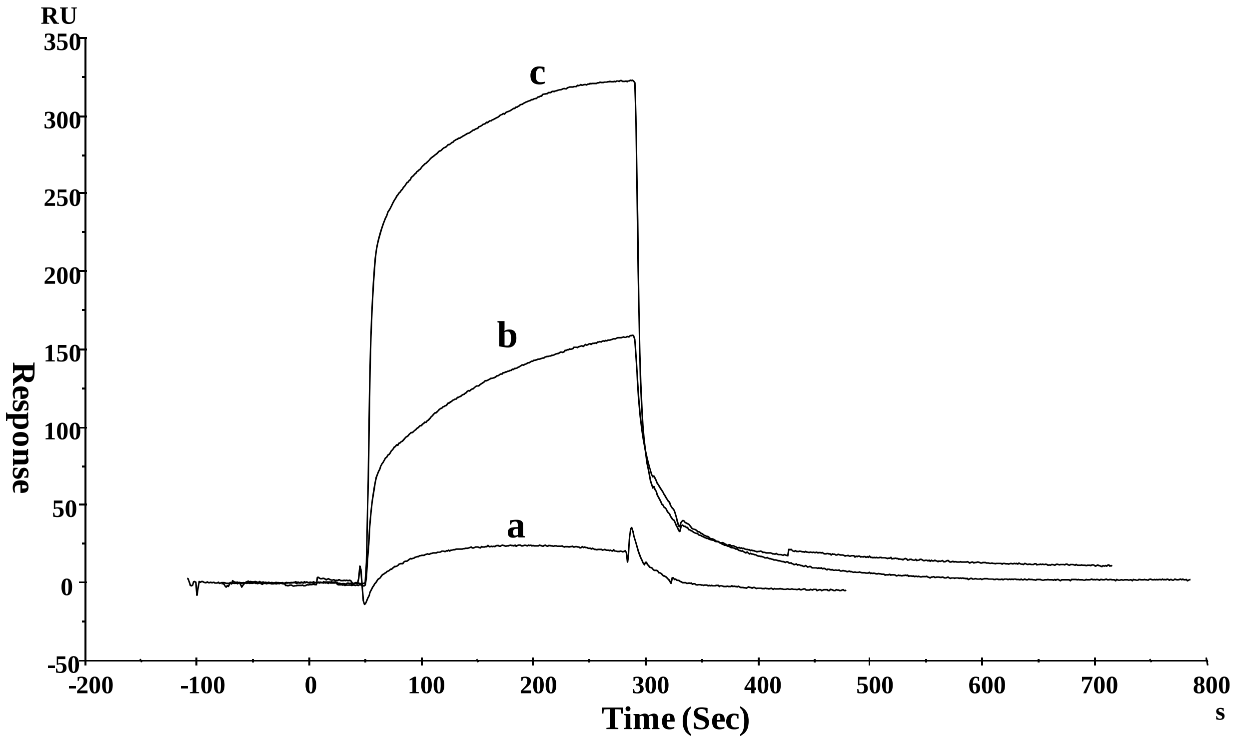
1550
1350
1150
950
750
550
350
150
-50
-200
0
200
400
600
800
1000
Time (Sec)
a
b
c

Figure S1 (B)


RU
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250
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100
50
0
-50
Response
c
b
a
-200
-100
0
100
200
300
400
500
600
700
800
s
Time (Sec)

Figure S2

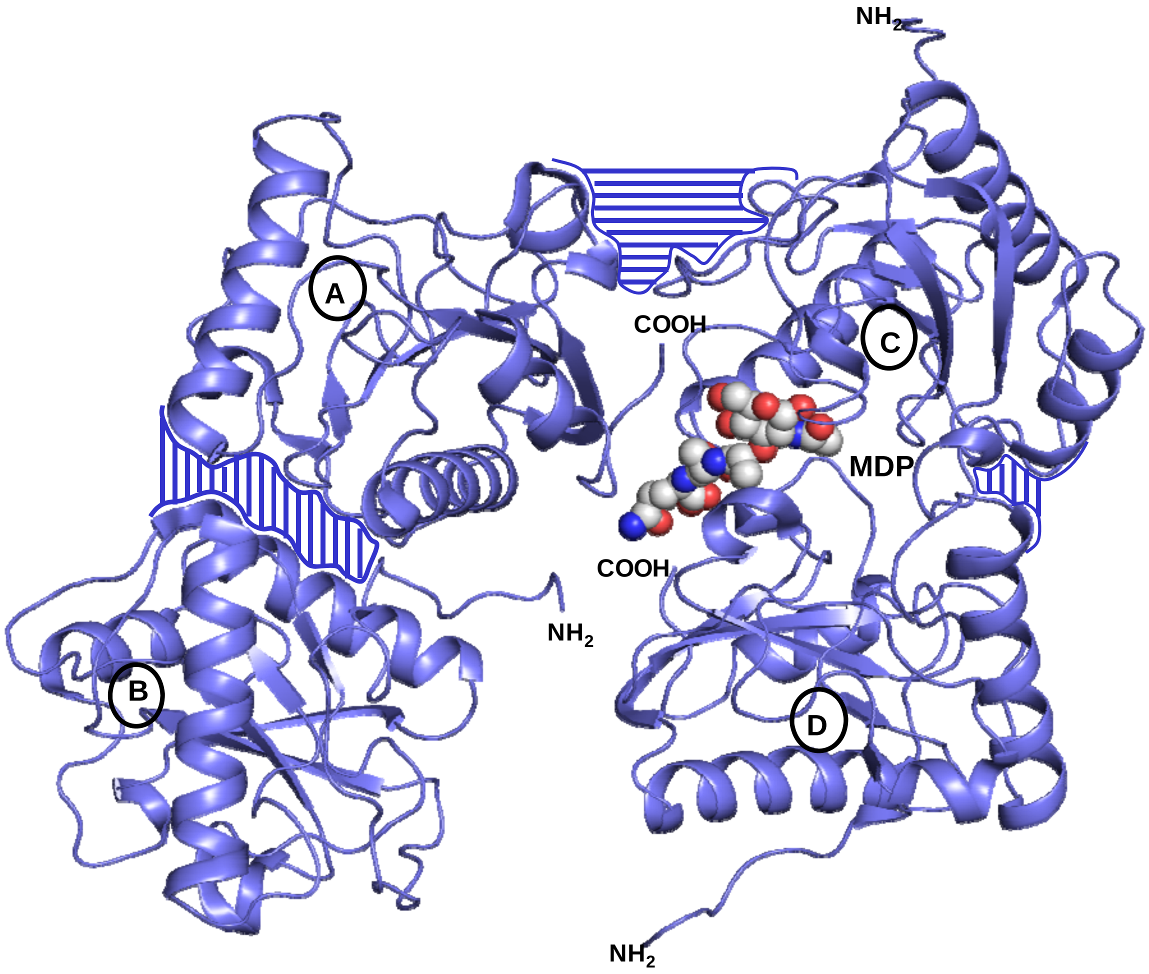

Figure S3

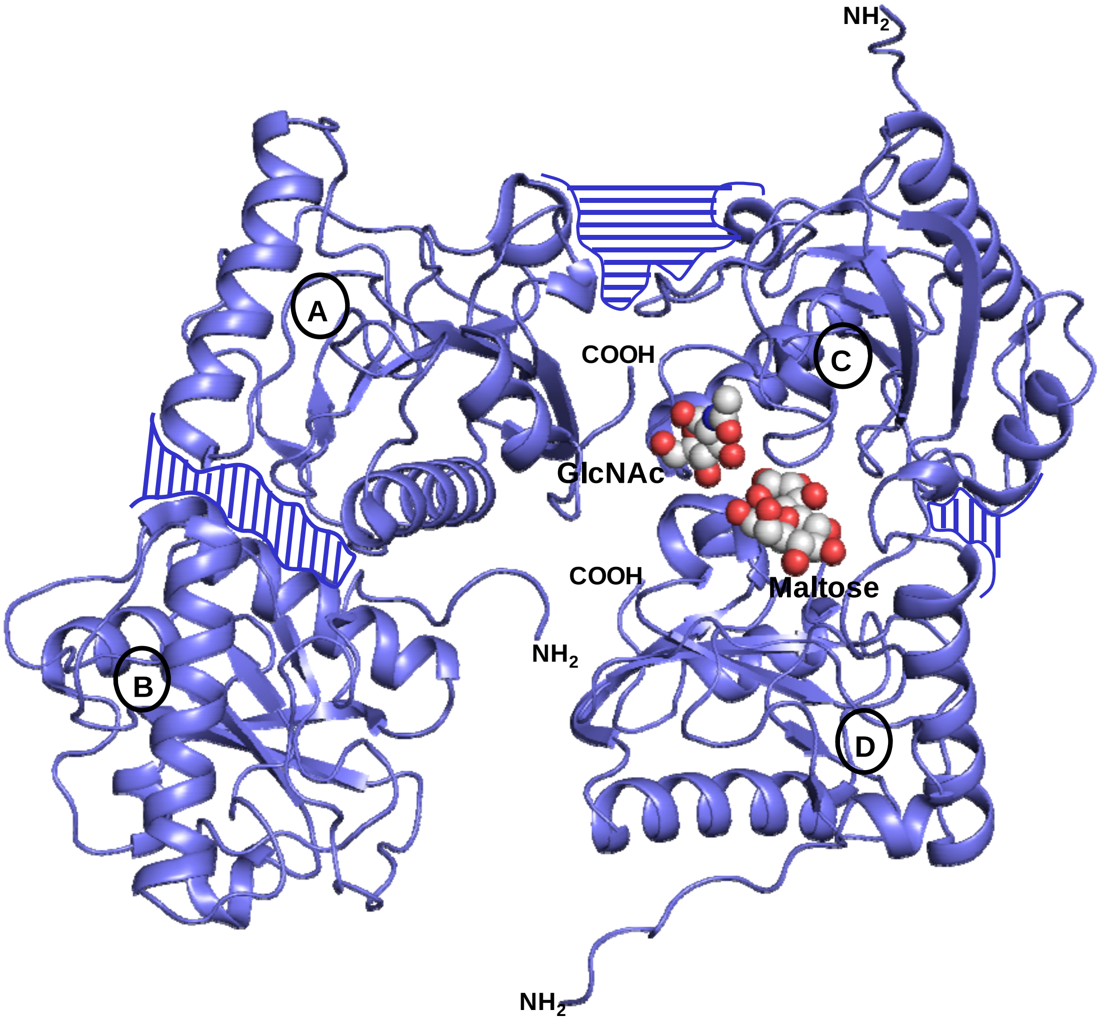

Figure S4

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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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Table S1: Hydrogen bonded interactions between CPGRP-S and MDP

PROTEIN/WATER	MDP/WATER	DISTANCE (Å)
(C) His93 N	O1	3.39
(C) Asn99 ND2	O1	2.57
(C) Gly95 N	O7	3.01
(C) Gln150 NE2	O7	3.50
(C) Asn99 ND2	O5	3.30
(C) Asn99 ND2	(C) OW192	2.81
(C) OW-192	O5	2.99
(C) Trp66 NE1	O18	2.70
(D) Gln150 OE1	O18	3.29
(D) Val149 O	O18	2.84
(C) Leu64 O	O19	3.00
(D) OW179	O6	2.66
(C) Arg85 NH2	(D) OW179	3.15
(C) Arg85 NH1	(D) OW248	3.43
(D) Thr97 OG1	N4	3.26
(D) Trp98 NE1	O22	3.41
(D) Asp148 O	O22	2.98
(D) Asp148 O	O23	3.02
(D) OW-188	O23	3.41
(D) Asp148 O	(D) OW188	2.98
(D) OW177	N20	3.21
(D) Lys144 NZ	(D) OW177	2.63

Table S2: Hydrogen bonded interactions between CPGRP-S and GlcNAc

PROTEIN/WATER	GlcNAc/WATER	DISTANCE (Å)
(D) OW182	O6	3.39
(D)Thr97 OG1	(D) OW182	2.98
(C) OW563	O6	2.77
(A) Asn140 ND2	(C) OW563	3.25
(A) OW277	O6	3.25
(A) Asn140 ND2	(A) OW277	2.88
(D) Gln150 OE1	O4	3.23
(D) Gln150 OE1	(D) OW183	3.15
(D) Ala94 O	(D) OW183	2.78
(C) Pro96 N	O7	3.03
(C) Asn99 ND2	N2	3.44
(C) Lys90 NZ	(C) OW334	2.56
(C) Gly91 O	(C) OW334	2.89
(C) OW535	O3	2.68
(C) Asn99 ND2	(C) OW535	2.73

Table S3: Hydrogen bonded interactions between CPGRP-S and Maltose

PROTEIN /WATER	MALTOSE/WATER	DISTANCE (Å)
(C) Gln150 NE2	O2'	2.80
(D) Asn99 ND2	O6'	2.87
(D) Trp66 NE1	O2	2.87
(D) OW558	O6'	2.68
(D) Lys90 NZ	(D) OW558	3.09
(D) OW639	O3	2.85
(D) Gly65 O	(D) OW639	3.22
(D) Asn99 ND2	O5'	3.36
(C) OW185	O3'	3.01