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Supporting Material

Mapping Conformational Transitions in the Cyclic AMP Receptor Protein: Crystal structure and Normal Mode Analysis of M. tuberculosis apo-cAMP Receptor Protein

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Table 1: Refinement parameters (Numbers in parentheses represent the highest resolution shell)

No. of subunits per asymmetric unit	2
No. of protein atoms	3328
No. of water molecules per asymmetric unit	38
Resolution range (Å)	33.3 – 2.9 (3.2- 2.9)
R_{cryst}(%)	22.3 (27.6)
R_{free}(%)	29.6 (37.2)
rmsd from ideal values	
Bond distances (Å)	0.004
Bond angles (°)	0.631
Average B-factor (Å^2)	
Overall chain A	53.8
Main chain A	52.8
Side chain A	55.2
Overall chain B	35.6
Main chain B	33.0
Side chain B	38.3
Water	30.7
Sulfate ion	48.8
Structure validation	
PROCHECK-Ramachandran plot (%)	
Core	91.6
Allowed	8.4
MolProbity	
Clash score (all atoms)	0.91 (100th percentile)
Clash score (B < 40)	0
Rotamer outliers (%)	2.31
C^{β} deviations No. > 25	0
Protein Data Bank code	3H3U

R_{cryst} = Σ||Fobs| - |Fcalc||/Σ|Fobs|, where Fobs and Fcalc are observed and calculated structure factors.
R_{free} = ΣT||Fobs| - |Fcalc||/ΣT|Fobs|, where T is a test data set of about 5% of the total reflections randomly chosen and set aside prior to refinement.

Table 2

Interactions less than 3.5 Å distance among the symmetry mates of the 3D0S DNA binding domain.

Source atoms	Target atoms	Distance	cell	symmetry
/1/B/ 178(GLU). / CD [C]	/1/A/ 87(GLY). / C [C]	3.44	233	X-1,Y,Z
/1/B/ 178(GLU). / OE1[O]	/1/A/ 87(GLY). / O [O]	3.10	233	X-1,Y,Z
	/1/A/ 87(GLY). / C [C]	3.47	233	X-1,Y,Z
	/1/A/ 44(GLY). / N [N]	3.31	233	X-1,Y,Z
/1/B/ 181(ALA). / CB [C]	/1/A/ 43(PRO). / CB [C]	3.37	233	X-1,Y,Z
/1/B/ 182(GLN). / CB [C]	/1/A/ 45(ASP). / OD1[O]	3.45	233	X-1,Y,Z
/1/B/ 182(GLN). / CG [C]	/1/A/ 45(ASP). / OD1[O]	3.38	233	X-1,Y,Z
/1/B/ 182(GLN). / NE2[N]	/1/A/ 106(ARG). / NH2[N]	3.10	233	X-1,Y,Z
/1/B/ 188(ARG). / NE [N]	/1/A/ 43(PRO). / CG [C]	3.44	233	X-1,Y,Z
/1/B/ 166(GLY). / O [O]	/1/B/ 41(GLY). / N [N]	3.39	323	-X,Y-1/2,-Z+1/2
	/1/B/ 41(GLY). / CA [C]	3.28	323	-X,Y-1/2,-Z+1/2
/1/B/ 170(ARG). / CZ [C]	/1/A/ 159(GLN). / NE2[N]	3.45	323	-X,Y-1/2,-Z+1/2
/1/B/ 170(ARG). / NH2[N]	/1/A/ 159(GLN). / NE2[N]	3.45	323	-X,Y-1/2,-Z+1/2
/1/B/ 174(ASP). / OD2[O]	/1/A/ 164(GLN). / N [N]	3.33	323	-X,Y-1/2,-Z+1/2
/1/B/ 200(HIS). / NE2[N]	/1/B/ 107(ASP). / OD1[O]	3.50	323	-X,Y-1/2,-Z+1/2
	/1/B/ 107(ASP). / OD2[O]	3.20	323	-X,Y-1/2,-Z+1/2
/1/B/ 202(GLY). / CA [C]	/1/B/ 44(GLY). / O [O]	3.38	323	-X,Y-1/2,-Z+1/2
/1/B/ 202(GLY). / O [O]	/1/B/ 87(GLY). / CA [C]	3.08	323	-X,Y-1/2,-Z+1/2
	1/B/ 44(GLY). / N [N]	2.99	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / C [C]	2.95	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / O [O]	3.23	323	-X,Y-1/2,-Z+1/2
	/1/B/ 43(PRO). / CA [C]	3.20	323	-X,Y-1/2,-Z+1/2
	/1/B/ 88(PRO). / N [N]	3.39	323	-X,Y-1/2,-Z+1/2
/1/B/ 203(TRP). / NE1[N]	/1/B/ 43(PRO). / CG [C]	3.34	323	-X,Y-1/2,-Z+1/2
/1/B/ 203(TRP). / CE2[C]	/1/B/ 43(PRO). / CG [C]	3.49	323	-X,Y-1/2,-Z+1/2
/1/B/ 204(ILE). / O [O]	/1/B/ 86(PRO). / O [O]	3.38	323	-X,Y-1/2,-Z+1/2

/1/B/ 205(ARG). / NE [N]	/1/B/ 85(ASP). / OD2[O]	3.08	323	-X,Y-1/2,-Z+1/2
/1/B/ 205(ARG). / NH1[N]	/1/B/ 85(ASP). / O [O]	3.16	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / N [N]	3.38	323	-X,Y-1/2,-Z+1/2
	/1/A/ 132(ARG). / CZ [C]	3.18	323	-X,Y-1/2,-Z+1/2
	/1/A/ 132(ARG). / NH1[N]	3.05	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / CA [C]	3.33	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / C [C]	3.32	323	-X,Y-1/2,-Z+1/2
	/1/B/ 87(GLY). / O [O]	3.49	323	-X,Y-1/2,-Z+1/2
	/1/B/ 88(PRO). / O [O]	3.46	323	-X,Y-1/2,-Z+1/2
/1/B/ 205(ARG). / NH2[N]	/1/B/ 88(PRO). / O [O]	3.03	323	-X,Y-1/2,-Z+1/2
/1/B/ 207(GLU). / OE2[O]	/1/A/ 129(ARG). / NH1[N]	3.25	323	-X,Y-1/2,-Z+1/2
/1/B/ 214(SER). / CA [C]	/1/B/ 41(GLY). / O [O]	3.41	323	-X,Y-1/2,-Z+1/2
/1/B/ 214(SER). / CB [C]	/1/B/ 41(GLY). / O [O]	3.05	323	-X,Y-1/2,-Z+1/2
/1/B/ 214(SER). / OG [O]	/1/B/ 42(GLU). / C [C]	3.48	323	-X,Y-1/2,-Z+1/2
	/1/B/ 43(PRO). / N [N]	3.33	323	-X,Y-1/2,-Z+1/2
	/1/B/ 88(PRO). / CG [C]	3.42	323	-X,Y-1/2,-Z+1/2
	/1/B/ 41(GLY). / O [O]	2.96	323	-X,Y-1/2,-Z+1/2

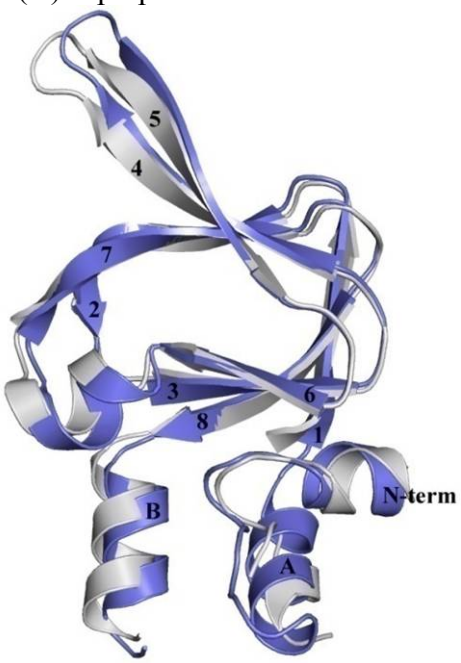
Table 3

Interactions less than 3.5 Å distance among the symmetry mates of the 3H3U DNA binding domain.

Source atoms	Target atoms	Distance	cell	symmetry
/1/B/ 178(GLU). / CD [C]	/1/B/ 178(GLU). / CD [C]	3.48	233	X-1,Y,Z
/1/B/ 178(GLU). / OE1[O]	/1/A/ 88(PRO). / CD [C]	3.43	233	X-1,Y,Z
/1/B/ 178(GLU). / OE2[O]	/ /1/A/ 88(PRO). / N [N]	3.43	233	X-1,Y,Z
	/1/A/ 88(PRO). / CA [C]	3.31	233	X-1,Y,Z
	/1/A/ 88(PRO). / CB [C]	3.35	233	X-1,Y,Z
	/1/A/ 88(PRO). / CG [C]	3.09	233	X-1,Y,Z
/1/B/ 182(GLN). / NE2[N]	/1/A/ 43(PRO). / CB [C]	3.47	233	X-1,Y,Z
/1/B/ 193(LYS). / CE [C]	/1/A/ 207(GLU). / OE1[O]	3.44	234	X-1/2,-Y+1/2,Z+1
/1/B/ 193(LYS). / NZ [N]	/1/A/ 207(GLU). / OE1[O]	2.73	234	X-1/2,-Y+1/2,Z+1

Figure 1: Least squares superposition of the cAMP-binding and DNA-binding domains of the two chains of CRP_{Mt}.

(A) Superposition of the cAMP-binding domains



(B) DNA-binding domains

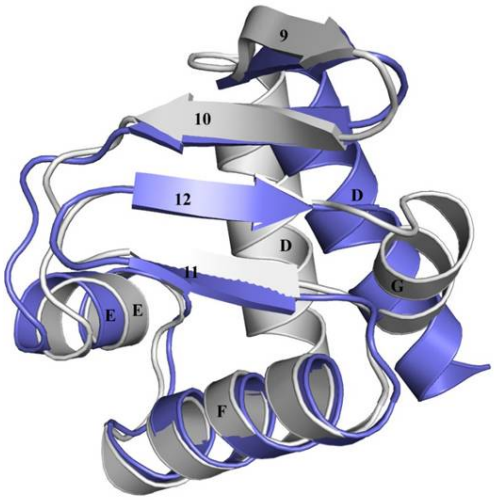
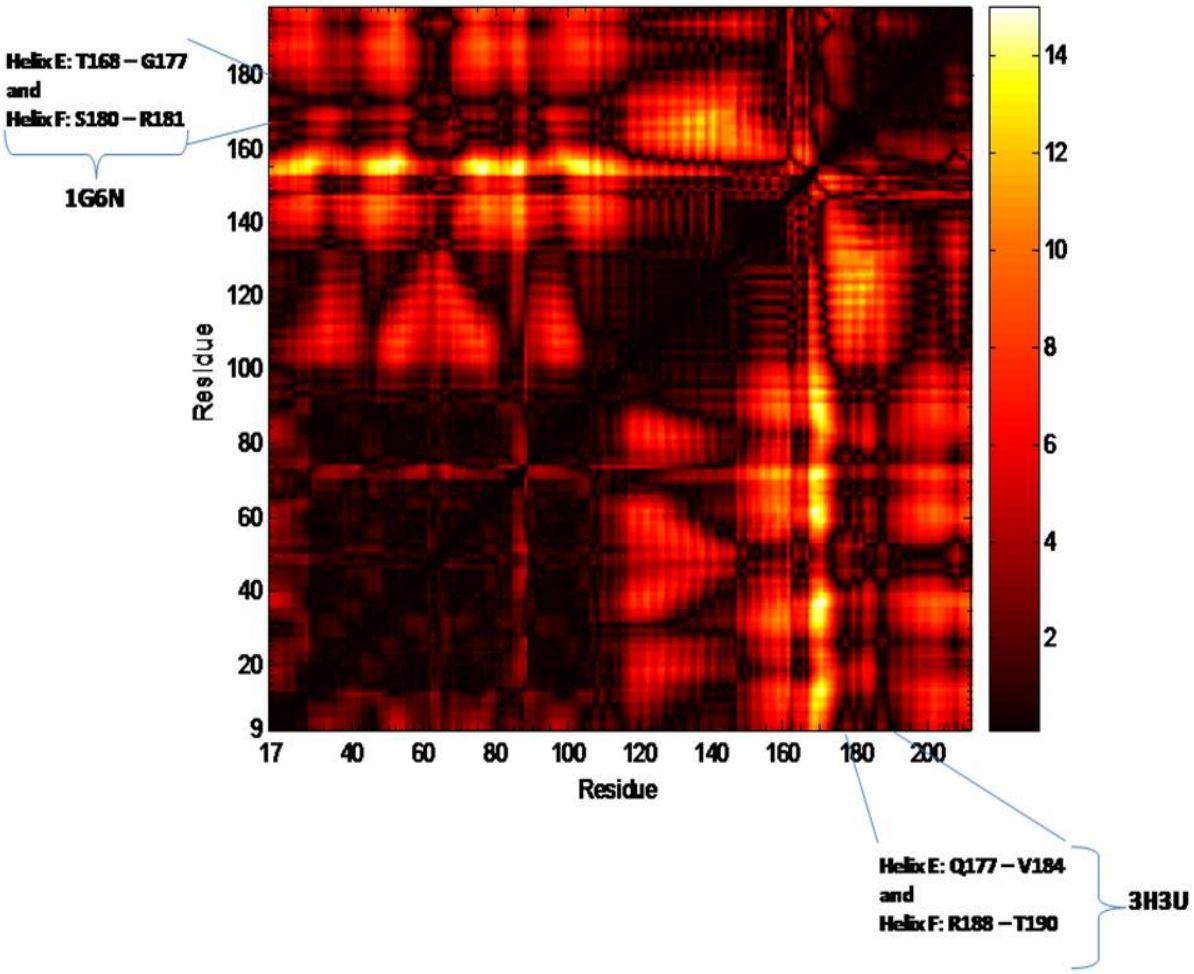


Figure 2: Analysis of conformational variations through difference distance maps

A. Difference distance map between the apo- structure (this report) and the holo- structure (PDB ID: 1G6N). Black shade represents smaller differences, while red and yellow shades represent larger differences. It can be clearly seen that along the diagonal of the map, the lower left corner, middle region and upper right corner show thickly populated black regions, implying that there are no major intra-domain conformational changes.

B. Difference distance map between the holo- structure (PDB ID: 1G6N) and the ternary complex (PDB ID: 1O3T). Color representation is the same as in A. Both the maps interestingly reveal that although the two domains have considerably different orientations, the last two turns of the E-helix and the first turn of the F-helix are invariant with respect to the cAMP-binding domain (see text for details).

A.



B.

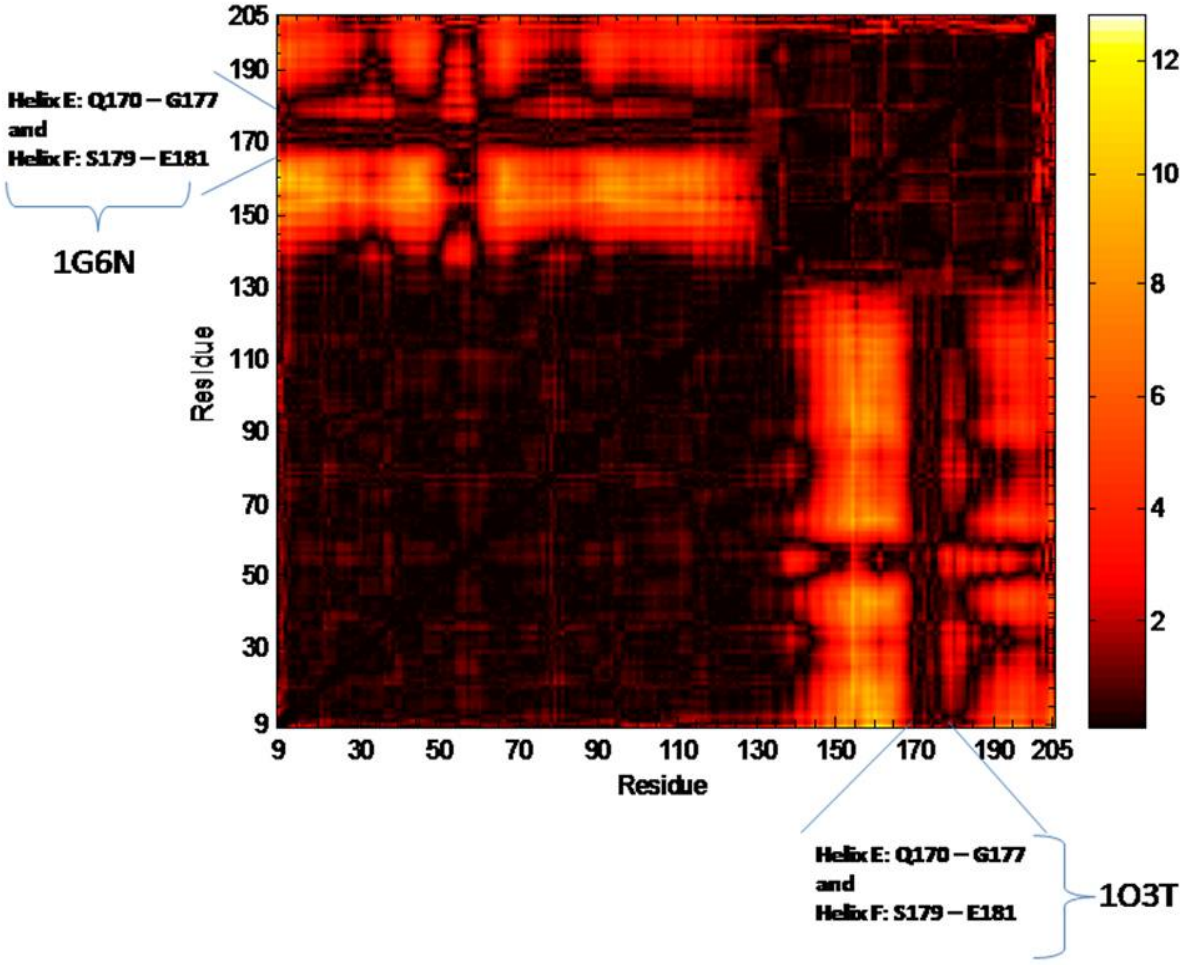
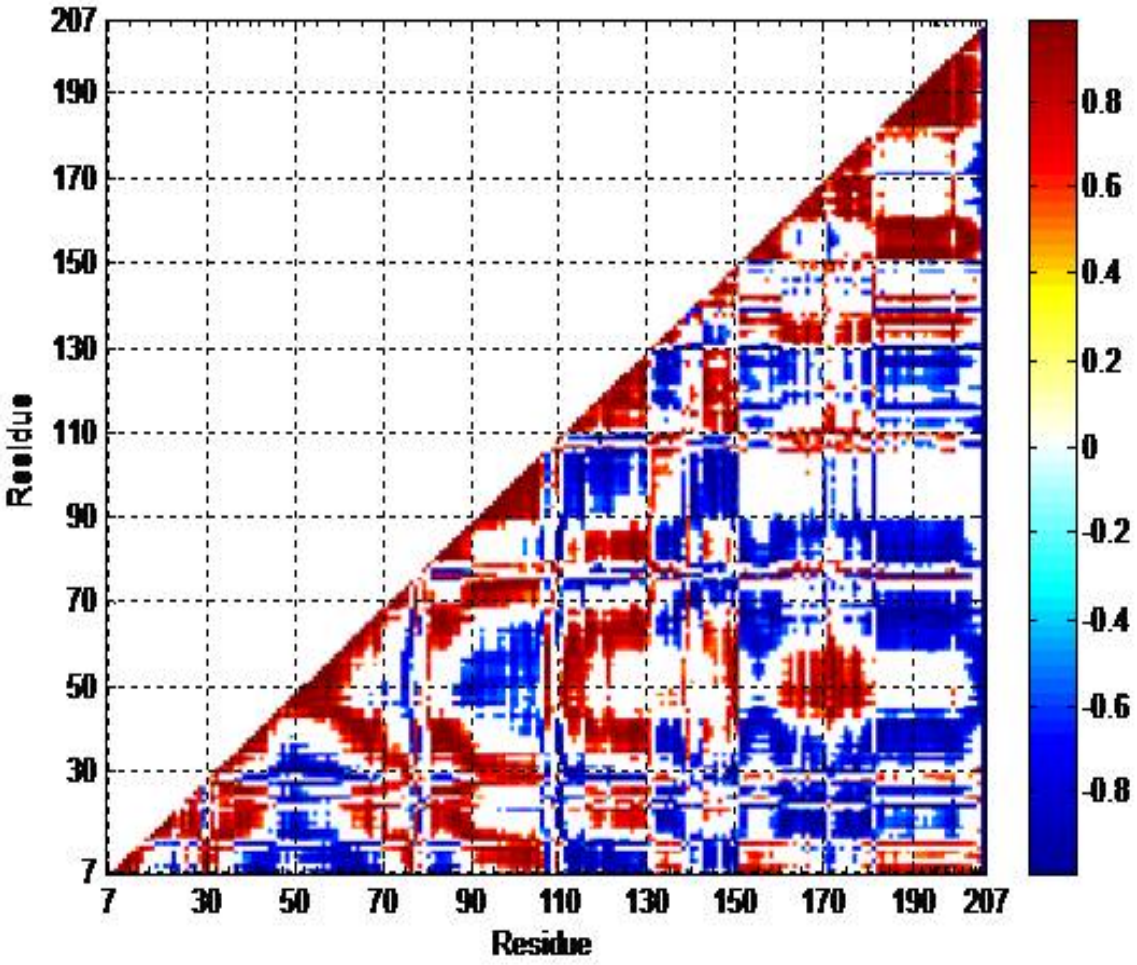

Helix E: Q170 – G177
and
Helix F: S179 – E181
1G6N
Residue
205
190
170
150
130
110
90
70
50
30
9
9
30
50
70
90
110
130
150
170
190
205
Residue
12
10
8
6
4
2
Helix E: Q170 – G177
and
Helix F: S179 – E181
1O3T

Figure 3

A. Correlation of motions in the *E. coli* structure. For these calculations, the *E. coli* holo- structure (1G6N) and the homology model of its apo- form were used. The residues which show correlated movements are shown in red, whereas those which show anti-correlated motions are shown in blue.

B. Correlation of motions in the *M. tuberculosis* structure. For these calculations, the *M. tuberculosis* apo- structure (this report) and the homology model of its holo- form were used. Colouring scheme is the same as that in (A). It is clearly seen that essential features of the two correlation matrices (A) and (B) are identical.

A.



B.

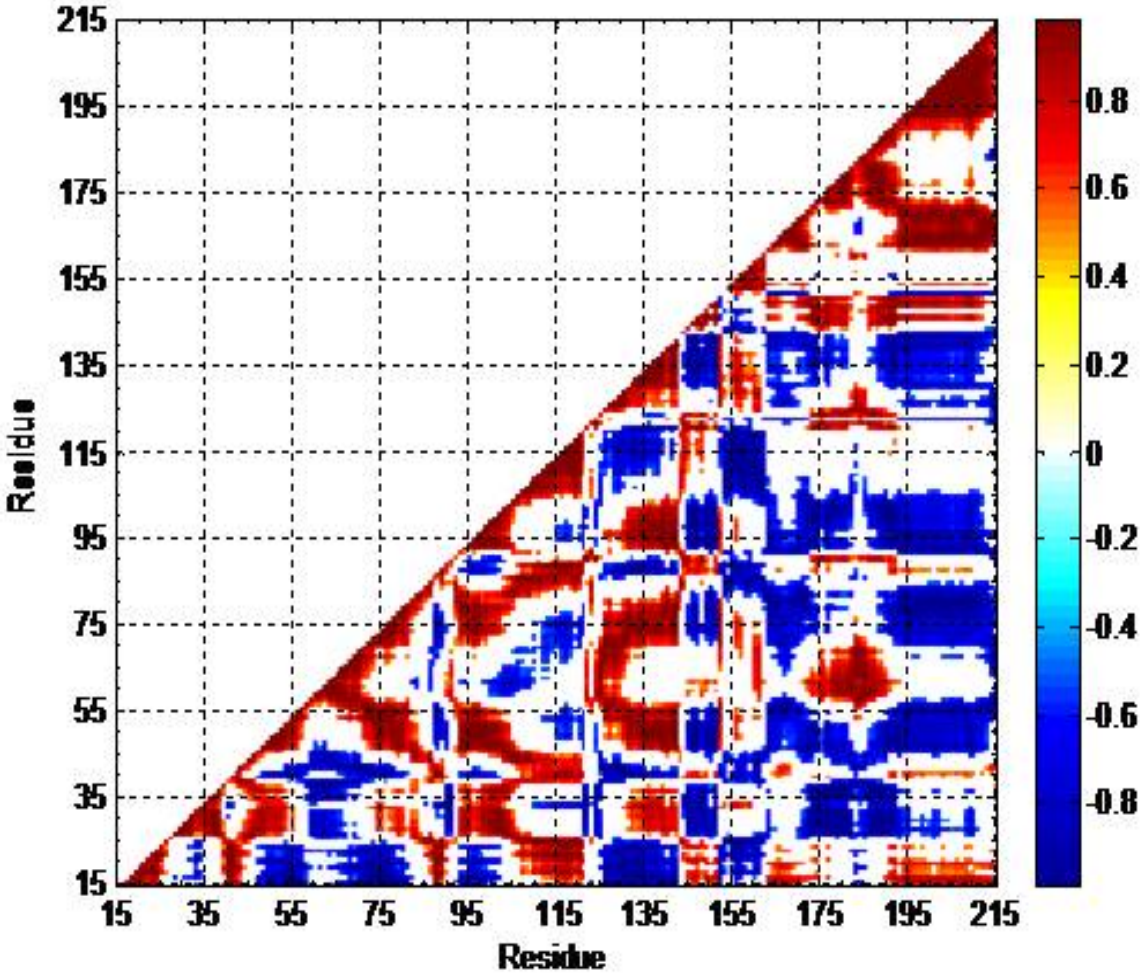

215
195
175
155
135
115
95
75
55
35
15
Residue
15 35 55 75 95 115 135 155 175 195 215
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8

Figure 4: **Overall structure of CRP_{Mt}.**

The labelling of secondary structural elements corresponds to the standard convention in the CRP structures of *E. coli*. Thus, the additional α-helix at the N-terminal observed in the *M. tuberculosis* structure has not been labelled. In the subunit shown in green colour, first 26 residues were found to be disordered, and thus this region was modelled in fragments, with only the most noticeable regions of electron density being accounted for.