An investigation to study the effects of cyclic-AMP to cAMP receptor protein from Mycobacterium tuberculosis by computational approach

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Abstract. The Cyclic AMP Receptor Protein of Mycobacterium tuberculosis, CRP$_{Mt}$ or Rv3676 is a global transcriptional regulator which shares several structural and functional features with the CRP protein of Escherichia coli. CRP$_{Mt}$ or Mtb CRP is also a homodimeric protein consists of an N terminal cAMP binding domain and C terminal DNA binding domain. In spite of having such high sequence identity with E. coli CRP, M.tuberculosis CRP differs in several aspects from this prototype. Moreover, it has also been reported that binding of cAMP to Mtb CRP induces a relatively small enhancement in specific DNA-binding by this protein. However, the main differences between CRP and CRP$_{Mt}$ become more apparent when comparing their functional dependence on cAMP binding. CRP cannot bind DNA in the absence of cAMP, which causes elaborate conformational changes that position the HTH domains for DNA binding. In contrast, CRP$_{Mt}$ shows specific DNA binding at most sites even in the absence of cAMP, although direct binding of cAMP to CRP$_{Mt}$ enhances its DNA binding affinity $\sim$2-fold. So, the significance of the presence of cAMP binding pocket in CRP$_{Mt}$ is still somewhat poorly understood. A long-standing question has been whether or not cAMP binding alone can cause CRP$_{Mt}$ to activate its cognate promoter. As an attempt to answer this question, we have done several docking experiments of Apo and ligand bound protein from MTb to its cognate promoter and analyze the results accordingly. We have also compared our docking experiment of Mtb CRP with E. coli CRP to have a wholesome idea about the conformational changes occurred by cAMP to Mtb CRP. Preliminary results from these studies are also included in this work.

1. Introduction

CRP/FNR (cAMP receptor protein/fumarate and nitrate reductase regulator) is one of the biggest members of a family of transcriptional regulators and its effector cyclic-AMP (cAMP) were discovered in E. coli during investigations to explain the phenomenon of diauxic growth more than 40 years ago. Since then, E. coli CRP–cAMP has become a paradigm of gene regulation, providing insights into signal perception and transduction, DNA recognition by regulatory proteins, regulator–polymerase interactions and promoter architecture. The Escherichia coli cAMP receptor protein, CRP, activates transcription at more than one hundred promoters. CRP functions by binding, in the presence of the allosteric effector cAMP, to specific DNA sites in or near target promoters and enhancing the
ability of RNA polymerase holoenzyme (RNAP) to bind and initiate transcription [1]. *Escherichia coli* CRP and FNR regulate transcription globally in response to glucose starvation and anaerobic conditions, respectively [2]. CRP has provided a classic model system for structural and mechanistic studies of transcription activation. Thus, CRP was the first transcription activator to have been purified [3, 4] and the first transcription activator to have its three-dimensional structure determined [5], and transcription activation by CRP has been the subject of extensive biophysical, biochemical, and genetic investigations [2, 6, 7].

In *E.coli* CRP, the N-terminal region houses the high-affinity cAMP-binding domain and the C-terminal region consists of a DNA-binding domain with a canonical helix-turn-helix motif. These two domains are connected by a long helix (C-helix) that forms a coiled-coil at the dimer interface and a short linker followed by another helix (D-helix) (Figure 1). Cyclic-AMP binding to the sensory domain is initially exothermic ($\Delta H_1 = -16.3 \text{ kJ mol}^{-1}; \Delta S_1 = 41 \text{ J K}^{-1} \text{ mol}^{-1}$) followed by an endothermic phase ($\Delta H_2 = 25.1 \text{ kJ mol}^{-1}; \Delta S_2 = 176 \text{ J K}^{-1} \text{ mol}^{-1}$) and cAMP interactions with the two protomers that make up the CRP dimer are cooperative ($\Delta G_2 - \Delta G_1 = 2.7 \text{ kJ mol}^{-1}$) with binding constants of $8 \times 10^4 \text{ M}^{-1}$ for site 1 and $6 \times 10^4 \text{ M}^{-1}$ for site 2. [8]. So, for *E. coli* CRP, cAMP binding is a cooperative process. In the apo-CRP dimer (without cAMP), the two DNA-binding domains interact to form a rigid body in which the DNA recognition helices are buried such that they cannot interact with DNA [9]. Binding of cAMP to CRP initiates structural rearrangements about a ‘hinge’ region allowing the DNA-binding domains to relocate relative to the cAMP- binding domain in a process mediated through hydrogen bonds between the N(6) position of adenosine with Ser- 128 of the dimerization helices (C-helices; Figure 1 & Figure 2) [9, 10]. According to the current understanding, cAMP-free CRP$_{EC}$ is unable to bind DNA with high affinity, or, alternately, it binds to nonspecific sequences. Upon binding to one cAMP molecule, it undergoes a conformational transition that is capable of binding DNA [2].

![Figure 1](image-url)
Although there is mounting evidence to suggest that the *E. coli* CRP undergoes allosteric conformational changes upon cAMP binding from a variety of spectroscopic and biochemical techniques, little is known about CRP of *Mycobacterium tuberculosis* or CRP<sub>Mt</sub>. In the year 2005, there was the first report on the characterization of Rv3676 protein (CRP<sub>Mt</sub>) using computational and experimental methods. It provided the first direct evidence for cAMP binding to a transcription factor in *M. tuberculosis*, thereby suggesting a role for cAMP-mediated signal transduction in this bacterium [11].

CRP<sub>Mt</sub> is a homodimeric protein that undergoes allosteric changes upon cyclic AMP binding and controls transcription of several genes involved in different physiological responses. Although a homolog of *E. coli* CRP, CRP<sub>Mt</sub> is different in several aspects. The *M. tuberculosis* CRP (Rv3676; 32% amino acid identity to *E. coli* CRP over 189 amino acids, including four of the six key cAMP-interacting residues in the sensory domain of *E. coli* CRP; Table 1) differs from the *E. coli* paradigm at several levels. The Rv3676 homodimer exhibits relatively weak (K<sub>b</sub> = 1.7 × 10^4 M<sup>-1</sup>) binding of cAMP to two independent sites (1:1 cAMP/protomer). Furthermore, cAMP-binding is endothermic (ΔH = 30.7 kJ mol<sup>-1</sup>; ΔS = 183 J K<sup>-1</sup> mol<sup>-1</sup>; ΔG = -23.7 kJ mol<sup>-1</sup>) and thus binding is entropically driven [12].

Table 1: Comparison of features of cAMP-signaling in *E. coli* and *M. tuberculosis*.

| BACTERIUM | *M. tuberculosis* | *E. coli* |
|-----------|------------------|----------|
| NICHE | Lung Macrophage | Mammalian Intestine |
| Number of Adenylyl Cyclase genes | 16 | 1 |
| Intracellular cAMP concentrations | High | Moderate |
| CRP | Rv3676 | CRP |
| cAMP–CRP interactions | Independent Binding | Cooperative Binding |
| K<sub>D</sub> for cAMP binding | ~60 μM | ~13-16 μM |
| Number of chromosomal binding sites | >70 | >378 |
For *E. coli* CRP, cAMP binding is cooperative; the first binding event is exothermic and the second is endothermic, and the sensory domain binding sites are saturated by micromolar concentrations of cAMP [13]. This is not the case for CRP$_{Mt}$ where the cAMP-binding sites are independent. The significant difference between the two proteins in the context of the cAMP binding of CRP$_{Mt}$ is the substitution of Ser-128 in *E. coli* CRP by Asn in CRP$_{Mt}$. This residue (Ser-128) makes a cross-subunit contact with cAMP in *E. coli* CRP; i.e. Ser-128 of subunit B interacts with cAMP bound at subunit A [14]. The differences in binding of cAMP by the *E. coli* and *M. tuberculosis* CRP proteins suggest that the signal transduction pathways that promote site-specific DNA binding might be different, and this might be reflected in the relatively small enhancement in DNA binding caused by addition of cAMP [12].

![Figure-3: Schematic diagram of hydrogen bonding contacts of the adenine groups of cAMP in the binding pockets of *E. coli* (A) and *M. tuberculosis* (B) CRP proteins (Taken from Stapleton et al., 2009).](image)

Fig. 3 shows that the N6 position of cAMP is able to donate two hydrogen bonds, which interact with the acceptor $\gamma$ oxygen atoms of Thr-127 of one subunit (subunit A) and of Ser-128 in the other subunit (subunit B) in *E. coli* CRP. The latter two atoms are constrained to act as acceptors because they in turn donate hydrogen bonds to the main chain carbonyl acceptors of residues 123 in subunit A and 124 in subunit B, respectively.

Although CRP$_{Mt}$ binds cAMP, this interaction induces a relatively small enhancement in specific DNA-binding by the protein. This is in sharp contrast to *E. coli* CRP, where the presence of cAMP enhances specific DNA-binding by several orders of magnitude. It has also been proposed that CRP$_{Mt}$ homodimer exhibits relatively weak binding of cAMP to two independent sites [12]. Unlike *E. coli*, which has only one adenyl cyclase, *M. tuberculosis* possesses at least 16 Class III adenyl cyclase-like proteins, including soluble and membrane-associated multi-domain proteins, suggesting that their catalytic activities (10 of the 16 have been shown to act as adenyl cyclases) can be regulated by extracellular and/or intracellular signals [15]. It has long been recognized that mycobacteria secrete cAMP, but it is only more recently that the capacity to intoxicate macrophages with cAMP has been recognized as a contributor to virulence. Interestingly, cAMP levels increase upon infection of macrophages by *E. coli* [16], and furthermore, addition of cAMP to cultures of *M. tuberculosis* causes changes in gene expression. Recently, Bishai and co-workers [17] showed that upon infection of macrophages, a bacterially derived cAMP burst promotes bacterial survival by interfering with host signaling pathways, but as well as influencing host regulatory networks, cAMP is also important in bacterial gene regulation. Thus, the synthesis (in particular by Rv0386) and secretion of cAMP are central features of *M. tuberculosis* pathogenesis and result in the bacterium being exposed to relatively high concentrations of cAMP. Rv3676 (CRP$_{Mt}$) was earlier reported to be essential for the survival of mycobacteria inside macrophages and in animal models [18]. CRP$_{Mt}$ and
its regulated genes are important in pathogenesis of mycobacteria and that these might have co-evolved with the pathogenic branch as a result of genome optimization aimed at devising new survival strategies. That many of these predicted target proteins are critical for the survival of the mycobacterium in the hostile environment of the macrophage [19]. The crystal structures of apo-Rv3676 and Rv3676-cAMP reveal that cAMP-binding is associated with much less dramatic structural rearrangements than those observed for *E. coli* CRP [20, 21, 22] (Figure 4). Consistent with the relatively mild structural rearrangements that occur upon cAMP-binding by Rv3676, the formation of the Rv3676–cAMP complex has a relatively small effect (almost 2-fold) on DNA-binding to a consensus sequence that is very similar to that of *E. coli* (GTGnnAnnnnnCACA) [18]. Furthermore, unlike *E. coli* CRP, apo-Rv3676 is capable of site-specific DNA-binding and transcription regulation [12].

![Figure 4: X-ray crystal structures of Mycobacterium tuberculosis CRPs in the (A) absence and (B) presence of cAMP](image)

It has already been observed that Mtb CRP is active even in the absence of cAMP, so the main question that could be asked that what would be the role of cAMP in *M. tuberculosis*? Moreover, *M. tuberculosis* is known to possess multiple adenylate cyclase gene, so it can be concluded that cAMP signaling should be a regular phenomenon in mycobacterial biology. Since all known effects of cAMP in prokaryotes are regulated by CRP or CRP like proteins, so a detailed study of Mtb CRP or CRPMt could be useful to elucidate the possible role of cAMP in *M. tuberculosis*. To understand this fact, the structure of Apo and ligand bound protein from *M. tuberculosis* to its cognate promoter need to be solved. But, answers to this question remain elusive, because there are only two crystal structures available and that of apo-CRP Mt and CRP Mt-cAMP. There is no crystal structure available of apo protein and CRP Mt-cAMP along with its cognate promoter or DNA. For the unavailability of these particular structures, we want to investigate, the effect of cAMP binding on the DNA binding properties of the protein.

On the other hand, the available crystal structure of cAMP-free CRP Mt structure (3D0S [20] and 3H3U [22]) has significant differences in the conformation of its two DNA binding domains. The most noticeable difference between the 3D0S structure and 3H3U structure, is in the helix-turn-helix motif of the B chains. In the 3D0S structure, the DNA-recognition F-helix is drawn toward the D-helix, which makes its conformation significantly different from that of any other reported structures (Figure 5).

It was also reported that the helix-turn-helix motif (the DNA binding domain) takes a conformation closer to the cAMP-bound conformation of CRP of *E. coli* (Figure 6) and the key feature of sequence-specific recognition of DNA lies in the flexibility of the DNA-binding domain only in the presence of the ligand, cAMP [22]. But previously it was reported that, unlike *E. coli* CRP, apo-Rv3676 is capable
of site-specific DNA-binding and transcription regulation [12] and Rv3676 can significantly influence gene expression without the need to bind cAMP [23].

Figure 5: Superposition of the B-chain of CRPMt (3H3U) and CRPMt (3D0S). (Taken from Kumar et al., 2010)

Figure 6: Superposition of the DNA-binding domains of the CRP Ec and CRP Mt. (A) Superposition of the DNA-binding domain of the cAMP-bound CRP Ec (cyan) and cAMP-free CRP Mt (green) (PDB ID: 3D0S). (B) Superposition of the DNA-binding domain of the cAMP-bound CRP Ec (cyan) and cAMP free CRP Mt (green) (PDB ID: 3H3U) (Taken from Kumar et al., 2010)

So now it is very difficult to confer that which structure of cAMP free CRP Mt is preferable. So, it can be only understood when both the structure of Apo CRP Mt and cAMP bound CRP Mt with its DNA, will be solved. For preliminary, we want to investigate the interaction of both these structures with its cognate DNA by Molecular Docking.

2. Materials & Method

2.1. Acquisition of sequences

Amino acid sequences of cAMP receptor protein of E. coli and Mycobacterium tuberculosis from were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) database.
2.2. Protein structures

All experimental crystal structures of proteins and DNA were collected from the Protein Data Bank (https://www.rcsb.org/). The information of all crystal structure of protein and DNA are given in Table-2. In the structures, all water molecules, ions, and ligands were removed from the original files using Text-Pad. Some parts of the structures like three letter codes of amino acids and nucleic acids, alignments, which need to be edited, will be edited by using text pad.

Table-2: The information of all crystal structure of protein and DNA used in experiment

| PDB File | Nature of complex | Depositor | Resolution (Å) | R value | Residue No (Active Residues) |
|----------|-------------------|-----------|----------------|---------|-----------------------------|
| 3D0S     | Apo CRP-MTB       | Gallagher, D.T., Robinson, H., Reddy, P.T. | 2       | 0.212              | 185-221 (A chain) 409-445 (B chain) |
| 3H3U     | Apo CRP-MTB       | Kumar, P., Joshi, D.C., Akif, M., Akhter, Y., Hasnain, S.E., Mande, S.C. | 2.9     | 0.223              | 185-221 (A chain) 409-445 (B chain) |
| 3HIF     | Apo CRP-E.coli    | Steitz, T.A., Sharma, H., Wang, J., Kong, J., Yu, S. | 3.59    | 0.295              | 180-207 (A chain) 381-408 (B chain) |
| dna_3MZI | DNA of M.tuberculosis (obtained from 3MZI*) | Akhter, Y., Wilmanns, M. | 2.9     | 0.247              | 5,6,7,16,17,18 (A chain) 26,27,28,37,38,39 (B chain) |
| pdb1cgp  | DNA of E.coli (obtained from 1CGP**) | Schultz, S.C., Shields, G.C., Steitz, T.A. | 3       | 0.235              | 9-24 (A Chain) 40-55 (B Chain) |

*3MZI- Crystal structure of cAMP receptor protein from mycobacterium tuberculosis in complex with cAMP and its DNA binding element
**1CGP- catabolite gene activator protein (cap)/dna complex + adenosine-3',5'-cyclic-monophosphate

2.3. DNA-protein docking studies

DNA-protein docking was carried out with HADDOCK (High Ambiguity Driven protein-protein DOCKing) (Vries et al., 2010) web server (HADDOCK2.2 @BonvinLab) (http://haddock.science.uu.nl/services/HADDOCK/haddock.php). The docking had been done in the easy interface. The docking study required two inputs, the Protein molecule and the DNA molecule. The Protein was the first molecule. The second molecule was the DNA. All chain of both structures was given for docking. The Active residues (directly involved in the interaction) were given. Define passive residues automatically around the active residues was selected.
2.4. Structural analysis of DNA-Protein complexes

After getting the docked structures, those structures are processed and validated using DNAProDB web-based visualization tool [27].

2.5. Multiple alignment

The sequences of Helix-turn-helix and F-helix of *E. coli* CRP and MtbCRP were aligned using *CLUSTALW* using the default parameters.

3. Result

The energy minimized structure models were used as the input for DNA-protein docking studies. Here in this study DNA strand of both organism, *Escherichia coli* and *Mycobacterium tuberculosis*, were taken from 1CGP (Catabolite gene activator protein (cap)/dna complex + adenosine-3',5'-cyclic-monophosphate) and 3MZH (Crystal structure of cAMP receptor protein from *Mycobacterium tuberculosis* in complex with cAMP and its DNA binding element) respectively. The consensus sequence of protein binding in *E. coli* and *M. tuberculosis* are TGTGANNNNNTCACA and GTGNNANNNNNCACA respectively. With the energy, minimized structures DNA-protein docking was completed by HADDOCK. HADDOCK easy interface was used to dock DNA and protein structure with the specified parameters. After a successful docking run, only top 10 clusters were displayed. From top 10 clusters the best one was chosen with the lowest HADDOCK score, lowest *Z*-score and also lowest RMSD value (Root Mean square deviation). For every cluster, various energies that make up the HADDOCK score were calculated and displayed like: electrostatic energy, van der Waals energy, desolvation energy, Restraints violation energy and a buried surface area (BSA) term. A HADDOCK score is a weighted sum of all these energy terms and also defined a rank to the structures after each docking stage [24]. The result of HADDOCK was tabulated in Table 3.

| Parameters                          | Apo CRP<sub>MT</sub> (3D0S) DNA | Apo CRP<sub>MT</sub> (3H3U) DNA | Apo CRP<sub>EC</sub> (3HIF) DNA |
|-------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| HADDOCK score                       | -95.8 +/- 14.8                  | -107.6 +/- 7.1                  | -67.9 +/- 9.3                   |
| Cluster size                        | 7                               | 26                              | 6                               |
| RMSD from the overall lowest-energy structure | 0.6 +/- 0.4                      | 13.4 +/- 0.1                    | 25.7 +/- 0.3                    |

Table-3: Docking Parameters of Apo CRP<sub>MT</sub> (3D0S) and Apo CRP<sub>MT</sub> (3H3U) with the DNA of *Mycobacterium tuberculosis* and Apo CRP<sub>EC</sub> (3HIF) with the DNA of *Escherichia coli*.
Table 3: Docking Parameters of Apo CRP<sub>MT</sub> (3D0S) and Apo CRP<sub>MT</sub> (3H3U) with the DNA of *Mycobacterium tuberculosis* and Apo CRP<sub>EC</sub> (3HIF) with the DNA of *Escherichia coli*.

The values of HADDOCK score and Z score along with RMSD and restrain violation energy indicates that the Mycobacterium Protein-DNA complex structure is more stable structure (Figure 7A) than that of *E. coli* (Figure 8). The other parameters like buried surface area was also granted to choose the best complex model as it describes about the difference between the sum of solvent accessible surface area (SASA) for each molecule and for the complex. In this result, buried surface area for CRP<sub>EC</sub> was 2912.2 +/- 301.5. But for CRP<sub>MT</sub> the buried surface area was 2780.3 +/- 115.4. The desolvation energy for CRP<sub>EC</sub> was 24.3 +/- 13.6. But for CRP<sub>MT</sub> the desolvation energy was 33.1 +/- 9.8. The best HADDOCK score and minimum RMSD value was seen in CRP<sub>MT</sub> protein - DNA complexes. CRP<sub>MT</sub> protein interacting with DNA gave the best result for complex structure with (-95.8 +/- 14.8) HADDOCK score (Fig 7A). The RMSD from the overall lowest-energy structure is 0.6 +/- 0.4 for this complex (Fig 7A). Thus, the total energy of CRP<sub>MT</sub>-DNA complex structure indicates a good stable structure (Fig 7A).

![Figure 7: Complex structure of DNA and CRP<sub>MT</sub> protein (A) 3D0S and (B) 3H3U. There are two monomers, one having yellow and green and other having cyan and blue color. The yellow and cyan colored helix area is mainly interacting with DNA strand. [25, 26, 27]](image-url)
The Z score of 3D0S and 3H3U are -2.0 and -1.4 respectively (Figure 7A & 7B). The desolvation energy for 3H3U was 22.9 +/- 8.0. But for 3D0S the desolvation energy was 33.1 +/- 9.8. The best HADDOCK score and minimum RMSD value was seen in 3D0S protein - DNA complexes. 3D0S protein interacting with DNA gave the best result for complex structure with (-95.8 +/- 14.8) HADDOCK score (Fig 7A). The RMSD from the overall lowest-energy structure is 0.6 +/- 0.4 for this complex (Fig 7A). Thus, the total energy of 3D0S CRPMT-DNA complex structure indicates that 3D0S is more strongly interacts with DNA than 3H3U (Fig 7A). Further validation was done using DNAProDB web-based visualization tool. Here, we got the buried solvent accessible surface-area (BASA) between individual residues and nucleotides. The BASA* value of 3D0S and 3H3U CRPMT-DNA complex are 1510.337 Å² and 1440.407 Å² respectively. Hydrophobicity scores for each protein residue in the protein surface are computed using the spatial aggregation propensity (SAP)* algorithm, with a 5.0 Å cut-off radius (Table 4) [27].

![Figure 8: Complex structure of DNA and CRP protein. There are two monomers, one having yellow and green and other having cyan and blue color. The yellow and cyan colored helix area is mainly interacting with DNA strand. [25, 26, 27]](image)

**Table 4: BASA value of 3D0S and 3H3U CRPMT-DNA complex are calculated and the Docked complex structures are validated using DNAProDB web-based visualization tool**

| DNA-protein Interfaces | DNA-protein Interfaces |
|------------------------|------------------------|
| **Mtb_CRP_3D0S_DNA**   | **Mtb_CRP_3H3U_DNA**   |
| DNA Entity ID          | DNA Entity ID          |
| Pro. Chain ID          | Pro. Chain ID          |
| Pro. Chain Segment     | Pro. Chain Segment     |
| Nuc-Res Interaction s  | Nuc-Res Interaction s  |
| Weak Nuc-Res Interaction s | Weak Nuc-Res Interaction s |
| Total BASA [Å²]        | Total BASA [Å²]        |
| Total Hbond s          | Total Hbond s          |
| Total vdw              | Total vdw              |
| Hydrophobicity Score (SAP) | Hydrophobicity Score (SAP) |
| Secondary Structure Compositio n | Secondary Structure Compositio n |
| B1[@B 2] A A1, A2 61 3 1510.33 7 17 150 -1.265 helix | B1[@B 2] A A1, A2 61 3 1510.33 7 17 150 -1.265 helix |
The Helix-turn-helix motif or more specifically the sequence of the F helix of both organisms are almost similar (Table 5).

The sequences of Helix-turn-helix and F-helix of *E. coli* CRP and MtbCRP were aligned using CLUSTALW using the default parameters.

| Helix-turn-helix  |  |
|-------------------|---|
| **CLUSTAL O(1.2.4) multiple sequence alignment**  |  |
| *E. coli* RQEIGQIVGCSRETVGRILKMLEDQNLI--------- 28  |  |
| *M. tuberculosis* SRETVNKLADFAHRGWIRLEGKSVLIS 28  |  |
| *F Helix*  |  |
| **CLUSTAL O(1.2.4) multiple sequence alignment**  |  |
| *E. coli* SRETVGRILKMLEDQNLI 18  |  |
| *M. tuberculosis* SRETVNKLADFAHRGWI 18  |  |

4. Discussion

In recent years, cAMP regulated gene expression in bacteria, is a major focusing area to understand CRP regulation in different bacteria. Changes in intracellular cAMP concentrations are perceived by CRP proteins that react with different sensitivities related to the niches occupied by the bacteria. Thus, the pathogenic *M. tuberculosis* Rv3676 is a low sensitivity CRP evolved to maintain some degree of responsiveness at the high cAMP concentrations used to intoxicate host macrophages; the commensal enteric bacterium *E. coli* possesses a mid-sensitivity CRP to regulate catabolite repression and chromosome structure, probably in response to energy charge; and the soil bacterium *P. putida* has a hypersensitive CRP, reflecting the very low concentrations of cAMP produced by this bacterium. At present, it appears that responding to the second messenger cAMP allows CRP to be co-opted to control different regulons in bacteria that occupy distinct niches. Thus, because cAMP intoxication of
the host is an important component of *M. tuberculosis* pathogenicity, its CRP has become desensitized to cAMP, whereas the CRP of the soil bacterium *P. putida* has become hypersensitive to cAMP [23].

But there are still many outstanding questions that need to be addressed to complete our understanding of cAMP-signaling in *E. coli* and other bacteria, especially in *Mycobacterium tuberculosis*. Firstly, *Mycobacterium tuberculosis* has its specific host (Human), and its niche extremely differ from *E. coli* and other bacteria. So, in case of *E. coli* CRP and CRP Mt, it may be a possible explanation that at the evolutionary context, *M. tuberculosis* got advantages for the substitution of Ser-128 to Asn at that position and this reduces the affinity and requirement of cAMP for activation of CRP Mt. Thus, the structure and regulation of CRP Mt differs from *E. coli* CRP.

Secondly, from our result, if the helix-turn-helix motif (the DNA binding domain) of 3H3U structure takes a conformation closer to the cAMP-bound conformation of *E. coli* CRP [22], then the docked structure of 3H3U structure with its DNA should be in a more stable form than that of 3D0S structure. But the values of HADDOCK score and Z score along with RMSD and restrain violation energy (Table 3) and the total BASA value (Table 4) clearly shows that the structure of 3D0S interacts more strongly with its DNA than that of 3H3U structure. So now it is very difficult to conclude that which structure of cAMP free CRP Mt or Apo-CRP Mt is preferable. So, further investigation needs to done. And, it can be only understood when both the structure of Apo CRP Mt and cAMP bound CRP Mt with its DNA, will be solved by Crystallography or NMR.

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