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Structure based mechanism of the Ca^{2+}-induced release of coelenterazine from the *Renilla* binding protein

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ABSTRACT

The crystal structure of the Ca^{2+}-loaded coelenterazine-binding protein from *Renilla muelleri* in its apo-state has been determined at resolution 1.8 Å. Although calcium binding hardly affects the compact scaffold and overall fold of the structure before calcium addition, there are easily discerned shifts in the residues that were interacting with the coelenterazine and a repositioning of helices, to expose a cavity to the external solvent. Altogether these changes offer a straightforward explanation for how following the addition of Ca^{2+}, the coelenterazine could escape and become available for bioluminescence on *Renilla* luciferase. A docking computation supports the possibility of a luciferase-binding protein complex.

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Key words: bioluminescence; EF-hand; coelenteramide; luciferase; Ca^{2+}-binding protein.

INTRODUCTION

Renilla luciferase is a bioluminescent protein that has found wide application, as a gene probe, for *in vivo* imaging, in diagnostic kits, and in other areas of biological research and biomedicine.[1] The popularity of such bioluminescence systems as probes results from the fact that the light emission and therefore the subject of the analysis, can be detected with extreme sensitivity.

Renilla luciferase catalyzes the molecular oxygen addition to coelenterazine, an imidazopyrazinone derivative, followed by its oxidative decarboxylation, to populate the product coelenteramide in its first electronic excited state. The blue bioluminescence, a broad band with maximum at 480 nm, originates from this fluorescence state of the coelenteramide. *Renilla* luciferase was first isolated from the Sea Pansy, *Renilla reniformis*, a soft coral found in shallow water marine environments.[2] The bioluminescence property probably functions to deter predators from nibbling at the polyps. Besides luciferase, the *in vivo* bioluminescence system localized within the photophore, involves two other proteins, the *Renilla* green-fluorescent protein, which modulates the color of the light emission, and a Ca^{2+}-triggered coelenterazine-binding protein (CBP).[3,4] The CBP is the same as the luciferin-binding protein (LBP) described by Charbonneau and Cormier.[3] When this protein was isolated from *R. reniformis* they called it "luciferin-binding protein" because the chemical structure of the *Renilla* luciferin had not been established at that time. As the chemical identification of the luciferin is now well established and to avoid confusion with unrelated luciferin-binding proteins such as the one involved in dinoflagellate bioluminescence,[5] we prefer "coelenterazine" as a rational replacement of the generic term "luciferin", that is the specific name "coelenterazine-binding protein". On disturbing the *Renilla*, the animal's nerve net induces a calcium ion flux setting off the bioluminescent flash over a time scale of tens of milliseconds. The role of CBP in this process evidently is 2-fold. As coelenterazine is unstable in free solution and as the membrane permeability of coelenterazine is rather high, these loss processes are prevented from occurring. CBP is an EF-hand protein[6,7] and its calcium binding property therefore provides the animal the ability to sense the presence of

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danger. On binding Ca^{2+}, the coelenterazine becomes available as a substrate for the luciferase and the bioluminescent flash response occurs. Possibly this reaction occurs in a luciferase-CBP complex so that the coelenterazine does not necessarily dissociate completely and be exposed to autooxidation in free solution.[7]

It is the purpose of our structure determinations to account for these properties of CBP. This work presents the structure of the apo-CBP with a Ca^{2+} loaded into each of the three consensus loops. The coelenterazine is not present in the binding cavity. By comparison with the CBP structure bound with coelenterazine[8] the mechanism of dissociation becomes apparent. On ligation of Ca^{2+}, shifts of certain helices create a "hole" thereby increasing the exposure of the binding cavity to solvent. Furthermore, residues in the binding cavity that were interacting with the coelenterazine are repositioned so that it becomes impossible for the coelenterazine to continue interacting simultaneously with all these original residues, thus weakening the affinity and allowing the molecule to protrude or escape through the opened hole. This conclusion is also supported by molecular docking of luciferase and apo-CBP loaded with Ca^{2+} according to which the "opened hole" in CBP lies opposite the putative *Renilla* luciferase active site.

METHODS

Cloning cDNA encoding CBP, expression in *E. coli* cells, and protein purification

The cloning and expression procedures have already been reported.[7] Briefly, the poly(A)+RNA was isolated from ~20 tentacles of three live specimens using the Straight A's mRNA Isolation System (Novagen). Clontech's SMART cDNA Library Construction Kit was used to synthesize the double strand (ds) cDNA from the poly(A)+RNA sample. The fragment of cDNA encoding CBP was obtained by performing PCR with degenerate primers. These primers were designed using the published amino acid sequence for CBP from *R. reniformis*.[6] Several isolated clones contained the 5′-ends of the CBP cDNA genes. From alignment of these isolated 5′-sequences the new primer complementary to the 5′-end of the CBP cDNA was designed and the full size cDNA gene for *R. muelleri* CBP cloned by PCR. For the bacterial expression of CBP, the coding sequence was amplified using specific primers and cloned for expression in a pET-19b vector (Novagen).

To produce protein for the structure determination of Ca^{2+}-loaded apo-CBP, the transformed *E. coli* cells (strain BL21-CodonPlus (DE3)-RIL (Novagen)) were cultivated with vigorous shaking in LB medium containing 200 μg mL^{-1} ampicillin at 37°C and induced with 1 m*M* IPTG when the culture reached an OD_{590} of 0.7–0.8. After induction the cultivation was continued for 3 h.

Table I
Summary of Crystallographic Statistics

Statistics	Refinement data	Phasing data
Data processing		
Resolution range (Å)	50.00–1.8 (10.00–1.8)	50.00–2.45 (2.54–2.45)
Wavelength (Å)	1.0	1.7
Space group	P 6_5	P 6_5
Cell dimensions (Å)	$a = b = 78.41$, $c = 125.00$	$a = b = 78.41$, $c = 125.00$
Unique reflections (free)	37,300 (2079)	29721 (1489)
Completeness (%)	100	98.57
I/σ (I)	38.77 (2.25)	39.5 (3.05)
R_{sym} (%)	15 (32)	7 (33.0)
Redundancy	6.7 (2.7)	13 (2.4)
Refinement		
No. of molecules/A.U.	2	2
Resolution range (Å)	10.00–1.8	10.00–1.72
R_{work} (R_{free})	20.00 % (25.00%)	19.88 % (22.89%)
Mean B factor (Å^2)	21.749	18.61
Protein atoms (solvent)	2798 (300)	1498 (188)
RMSD bond lengths (Å)	0.016	0.018
RMSD bond angles (°)	1.459	1.314

Protein purification

Most of the apo-CBP produced in the *E. coli* accumulates in inclusion bodies that can be easily isolated by centrifugation. The purification was performed as previously reported for recombinant obelin.[9] The apo-CBP sample in 6*M* urea obtained after chromatography on DEAE Sepharose Fast Flow was refolded by a 10-fold dilution in 20 m*M* Tris-HCI pH 7.1 with 1 m*M* $CaCl_2$ and purified by ion-exchange chromatography on DEAE-10 (Bio-Rad). Then the Ca^{2+}-loaded apo-CBP was concentrated and the chromatography buffer was replaced by 1 m*M* calcium acetate, 10 m*M* Bis-Tris pH 6.5. The protein concentration for crystallization was 13.5 mg mL^{-1} (Bio-Rad assay).

Crystallography

Initial screening for crystallization was carried out under 384 commercial conditions and by other methods described elsewhere.[10] The crystals of Ca^{2+}-loaded apo-CBP were grown from 0.1*M* HEPES pH 7.5, 2% PEG 400, 2.0*M* ammonium sulfate over less than 5 days incubation at 4°C. The crystals were frozen in liquid nitrogen in a cryoprotectant solution of 4.0*M* Li_2SO_4. The diffraction data were collected at 1.7 Å for phasing and 1.0 Å for refinement separately at beamline 22ID of the Advanced Photon Source. A continuous sweep of 360 consecutive 1° oscillation images was recorded with a Mar300 CCD detector at a crystal-to-detector distance of 180 mm for refinement data and 150 mm for phasing data separately, with an exposure of 2 s per image. Data reduction was carried out with the HKL2000 suite[11] (Table I). Initial phases were calculated by the Ca-SAS method with the program SOLVE/RESOLVE[12,13] incor-

porated in the high throughput structure determination pipeline Sca2Structure.[14] Automated model building was carried out with ARP/wARP.[15] Iterative model validation, rebuilding and refinement were carried out with MOLPROBITY,[16,17] XFIT[18] and the CCP4 program REFMAC5,[19] respectively. ARP/wARP and CCP4 programs were controlled through the CCP4I interface.[20] The final refinement statistics are shown in Table I. Coordinates of the refined model were deposited in the Protein Data Bank (PDB)[21] with access code 2HQ8.

Molecular docking

The model structure of *R. muelleri* luciferase was built using the three-dimensional structure of *R. reniformis* luciferase[22] (PDB code 2PSD) as a template (Web-server ESyPred3D: http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/). This template shares 91.8% identity with the *R. muelleri* luciferase sequence. The molecular docking was carried out with the use of the PatchDock program[23] which algorithm is based on shape complementarity principles (Web-server: http://bioinfo3d.cs.tau.ac.il/PatchDock/patchdock.html). For the docking computation request, the default conditions were specified: 4 Å for the "Clustering RMSD" parameter, any type of configuration for the complex, and no specific residues indicated as possible interaction sites.

RESULTS AND DISCUSSION

Overall structure

Figure 1 demonstrates that the structure of the apo-CBP with three bound Ca^{2+} and lacking the coelenterazine in the binding cavity, has the same compact scaffold and characteristic two-domain fold as the CBP itself before the addition of Ca^{2+}. In Figure 1(A) calcium atom is seen located in each of the canonical Ca^{2+}-binding sites, EF-hand loops I, III, and IV. Although the two proteins share the same overall fold, there are easily discerned local structure differences in almost every part, both in the helical and the loop regions. The RMSD of the Cα atoms between the two conformations is 3.91 Å. However, it should be noted that Ca^{2+} binding induces a greater conformational change in the N-terminal domain than at the C-terminus (Fig. 1, Table II).

Interactions between EF-hand motifs in N- and C-terminal domains

In the family of EF-hand proteins, the EF-hand motif almost always occurs in pairs. This double motif appears to be important for correct structural folding, and is thought to increase the affinity of each EF-hand for calcium.[24] Figure 2(A,C) shows that in CBP, the paired EF-hands display extensive hydrophobic interhelical interactions and a short β-type interaction between the two binding loops.

The EF-hand motif I of CBP is paired with EF-hand motif II, the latter having the characteristic structural features of an EF-hand motif but not the canonical sequence in the loop to permit calcium binding. The two loops of EF-hand motifs I and II also show typical interaction [Fig. 2(A)]. Loop I is bound with the loop II by means of hydrogen bonds between main chain nitrogen and carbonyl oxygen atoms of Ile31 and Ile76, main chain carbonyl oxygen and nitrogen atoms of Gly29 and Val78, the side chain $O^{\delta 1}$ of Asp28 and the side chain nitrogen atom of Arg75, and between N^{ζ} of Arg33 and the main chain carbonyl oxygen atom of Leu70 [Fig. 2(A)]. In Ca^{2+}-loaded apo-CBP [Fig. 2(B)], the hydrogen bond pattern of loop-loop interaction is practically the same; the binding of calcium ion abolishes only the hydrogen bond between the side chain $O^{\delta 1}$ of Asp28 and the side chain nitrogen atom of Arg75.

The loops of EF-hand motifs III and IV interact with each other as well both in the Ca^{2+}-free and in Ca^{2+}-loaded states of CBP [Fig. 2(C,D)]. The interaction occurs through hydrogen bonds between main chain nitrogen and carbonyl oxygen atoms of Val119 and Ile155. These hydrogen bonds are found both in Ca^{2+}-free and in Ca^{2+}-loaded states of CBP. In the Ca^{2+}-free state of CBP, there is a hydrogen bond between the main chain carbonyl oxygen atom of Gly117 and the main chain nitrogen of Arg157, which gets lost on binding of calcium ions into these loops.

Interhelical angles

The conformational changes that result from calcium binding to loops of the EF-hand motifs are usually described by the interhelical angles that represent more complex rearrangements of the interfaces between the α-helices of the domains.[25] Although such a description of Ca^{2+}-induced conformational transients is available only for a few Ca^{2+}-binding proteins, it provides a useful framework within which different types of conformational changes can be examined. For instance, the EF-hand motifs of "calcium-sensor class" proteins calmodulin and troponin C, are approximately anti-parallel in the Ca^{2+}-free state, but they become nearly perpendicular in the Ca^{2+}-loaded state.[25] This reorientation of α-helices exposes a hydrophobic patch, which is the basis of the biological function of these two proteins in living cells.

Unlike calmodulin or troponin C, the interhelical angles in CBP are almost perpendicular already in its Ca^{2+}-free state and Ca^{2+} binding does not change these angles significantly (Table III). In comparison to the effects in calmodulin and troponin C, we can conclude that the α-helices of CBP are optimally oriented for calcium binding. Nevertheless the movements of these helices on Ca^{2+} binding although small (Table III), appear

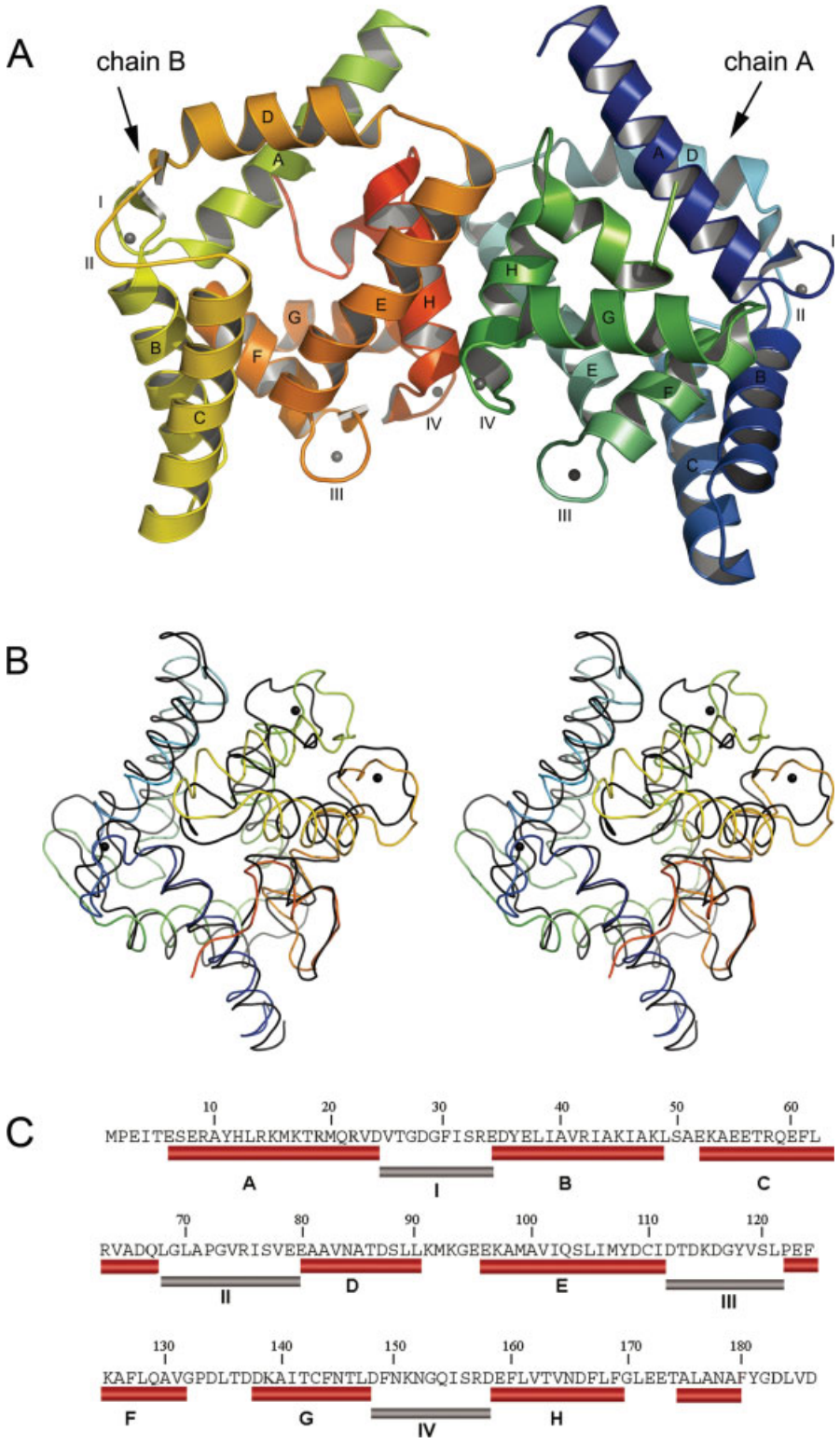

Figure 1

A. The crystal structure of *R. muelleri* Ca^{2+}-loaded apo-CBP. The protein crystallized as a dimer (the chains are designated by arrows, helixes are in multicolor). **B**. Stereoview of the superposition of *R. muelleri* CBP (the bound coelenterazine not included) (PDB code 2HPS, multicolor) and the Ca^{2+}loaded apo-CBP (PDB code 2HQ8, black). Calcium ions are shown as the gray balls in both cases. **C**. Primary structure of CBP; red bars correspond to the helices and gray the EF-hand loops.

Table II
RMS Deviation Values of Ca^{2+}-loaded apo-CBP versus CBP Bound with Coelenterazine

Structural parts of CBP	RMSD of Cα atoms (Å)
Overall	3.91
N-terminal domain	3.09
C-terminal domain	1.83
Loop I	1.68
Loop II	2.16
Loop III	0.95
Loop IV	0.62

to be sufficient to reduce solvent exposure of the coelenterazine on one side of the structure but also to induce the formation of a "hole" on the other side, an "escape route" for the molecule. This is shown by the surface model (see Fig. 3) where the CBP has solvent exposure at the 6-(*p*-hydroxyphenyl) position that then closes up to form the "escape hole" at the other end of the coelenterazine, at the 2-(*p*-hydroxybenzyl) substituent (see also Fig. 5). It is interesting that this region of the molecule is also the site of oxygen addition and it would seem very desirable that the luciferase should be nearby to efficiently capture coelenterazine before it would be lost through autooxidation.

Calcium-binding loops

Strynadka and James[24] proposed that conformational "pre-forming" of the ligands in a Ca^{2+}-binding loop would decrease the energetic cost in ordering this site for optimal Ca^{2+} binding. Among the Ca^{2+}-binding sites of CBP, the residues in Ca^{2+}-binding loops III and IV are found to be in this pre-formed state; they have to adjust only a little to accommodate a calcium ion. The RMSD between Ca^{2+}-loaded and Ca^{2+}-free states of the residues of these loops are 0.95 and 0.62 Å, respectively (Table II).

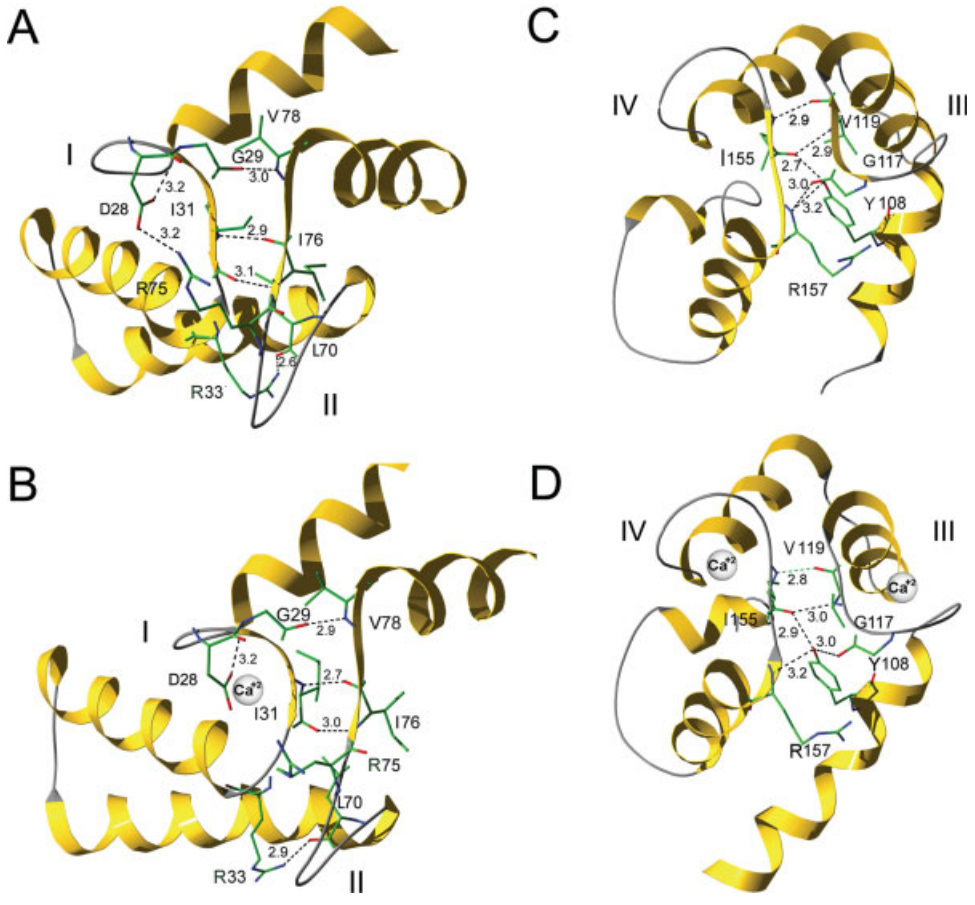

Figure 2
Small shifts of helix interactions in CBP (**A**, **C**) on Ca^{2+} binding (B, D) are typical of EF-hand proteins of the "Ca^{2+}-signal modulator" class.[25] A and **B** are the N-terminal EF-hands where one Ca^{2+} locates into loop I, and C and **D** are at the C-terminal with one Ca^{2+} binding to loops III and to IV. The dashed lines are inferred H-bonds and numbers are Å separation of donor and acceptor.

Table III
Interhelical Angles and Distances[a]

α-helices	Interhelical angle (CBP/Ca^{2+}-loaded apo-CBP)	Interhelical distance[b] (CBP/Ca^{2+}-loaded apo-CBP) (Å)
A–B	67.534/65.146	22.984/24.313
C–D	95.877/68.199	17.945/24.864
E–F	106.853/101.210	17.020/17.781
G–H	101.584/101.001	14.979/14.991

[a]The interhelical angles and distances were calculated using interhlx (K. Yap, University of Toronto).
[b]The distance between midpoints of the corresponding α-helices.

As a consequence, the Ca^{2+}-binding loops III and IV should have a higher affinity for calcium than does Ca^{2+}-binding loop I, for which the RMSD between Ca^{2+}-loaded and Ca^{2+}-free states is 1.68 Å. This higher RMSD might be a result of the twelfth position in loop I [D35, Fig. 4(A)], being occupied by Asp instead of the invariant Glu, as in canonical Ca^{2+}-binding consensus sequences. Also this Asp residue maintains a bidentate Ca^{2+} coordination, leading to a contraction of the loop. So far, among numerous EF-hand proteins, a similar substitution of Glu in the twelfth position to Asp is found only in sarcoplasmic Ca^{2+}-binding proteins.[26]

Figure 4 shows how the 12 residues of the EF-hand Ca^{2+}-binding loops (I, III and IV) shift their positions when going to the Ca^{2+}-loaded states. In all these loops, typical geometrical arrangement of the ligating oxygen atoms in a pentagonal bipyramid is observed, with the Ca^{2+} ion occupying the center of the pyramid. Also in each there are six oxygen ligands derived from the car-

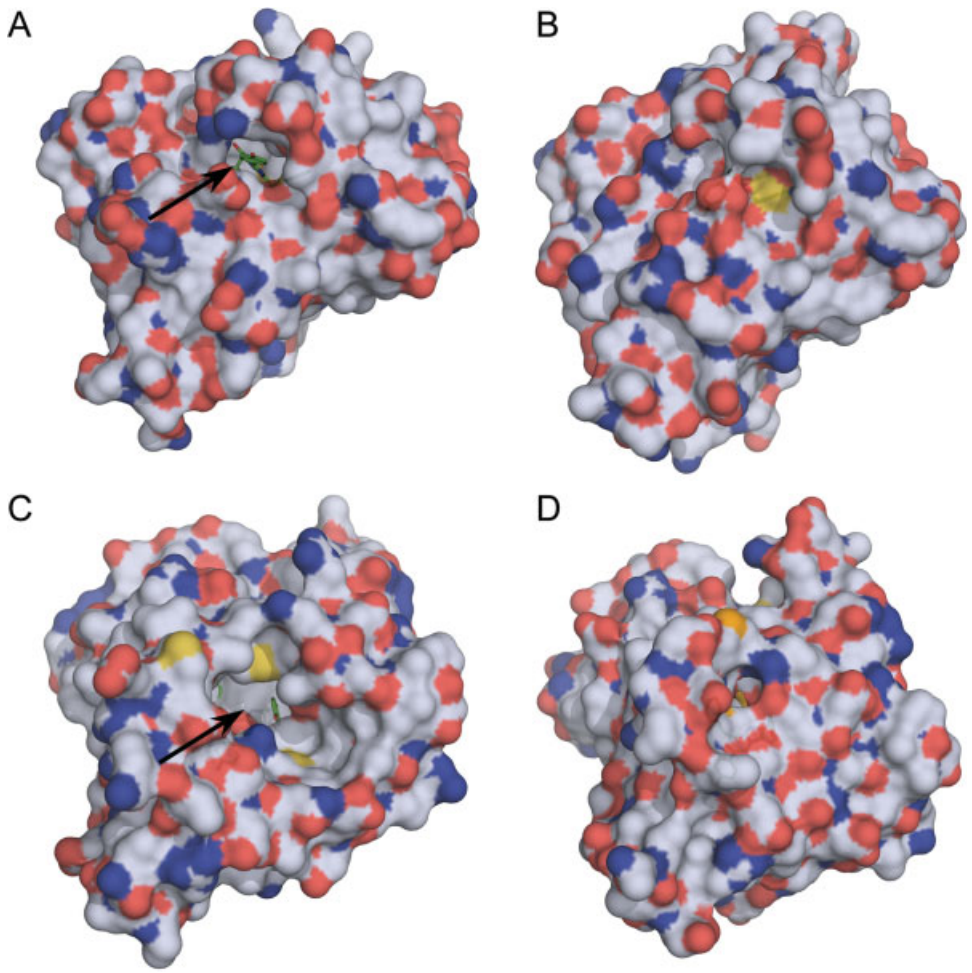

Figure 3
Molecular surface representation of CBP in Ca^{2+}-free and Ca^{2+}-loaded states. **A**. CBP loaded with coelenterazine (the green stick model), which can be seen through a solvent exposed opening (arrow). **B**. apo-CBP loaded with Ca^{2+} in the same orientation as CBP in (A), to show that the opening has closed up. **C**. apo-CBP loaded with Ca^{2+} viewed from the other side of the structure shows a new "hole" (arrow) exposing the cavity. **D**. CBP loaded with coelenterazine in the same orientation as apo-CBP loaded with Ca^{2+} in (C), to show that this "hole" is lacking before Ca^{2+} binding. The coelenterazine molecule is not actually present in the apo-CBP structure, but is placed there for the purpose of superimposing the CBP and apo-CBP structures. The white color represents carbon atoms, blue - nitrogen, red - oxygen and yellow - sulfur.

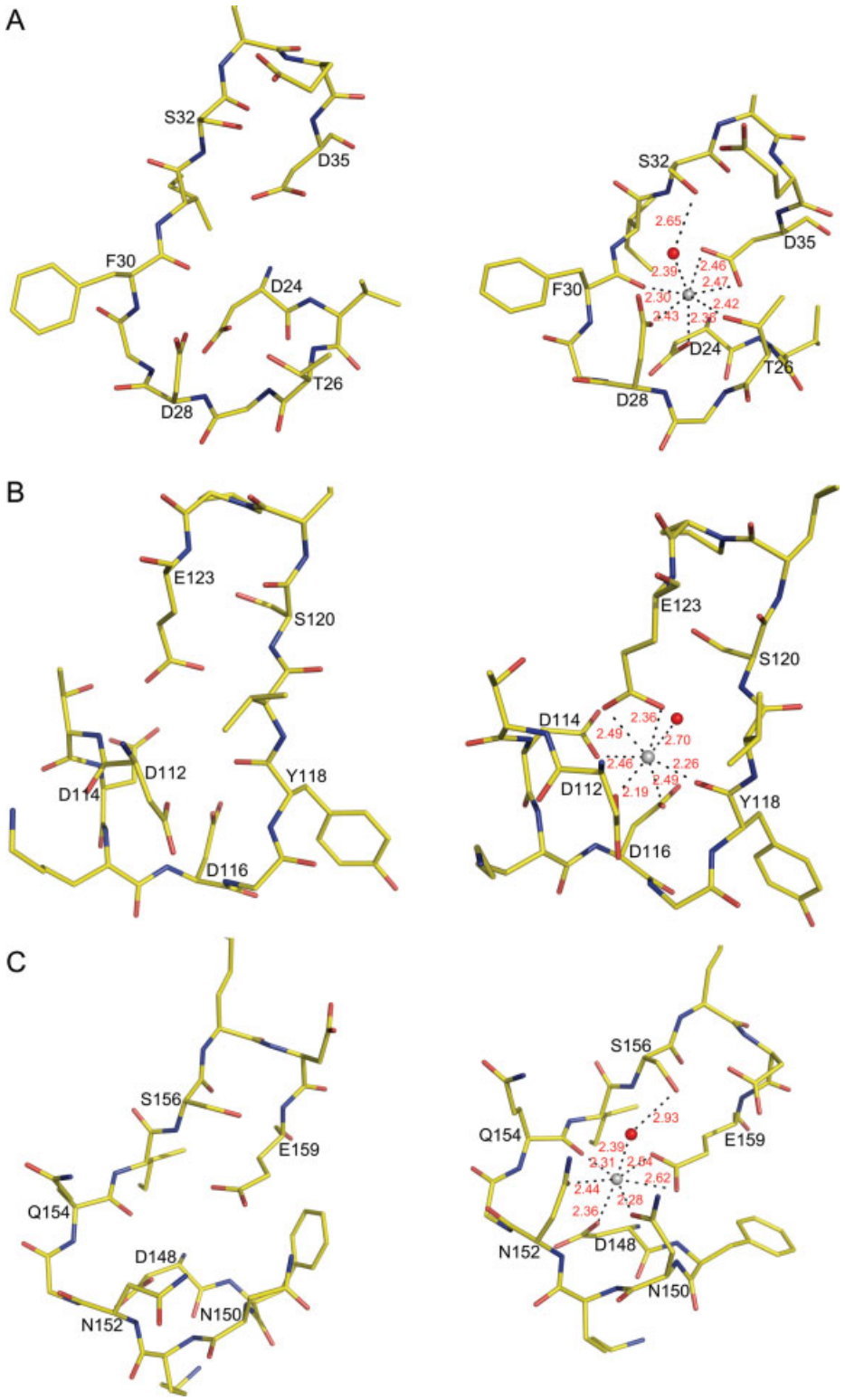

Figure 4

Structure of the Ca^{2+}-binding loops in the Ca^{2+}-free (left; PDB code 2HPS) and Ca^{2+}-loaded (right; PDB code 2HQ8) states. **A**, **B**, and **C** are the Ca^{2+}-binding loops I, III, and IV respectively. Calcium ions and water molecules are shown as gray and red balls, respectively. Distances are in Å. Ca^{2+}-binding loops in the Ca^{2+}-free and Ca^{2+}-loaded states are shown in the same orientation.

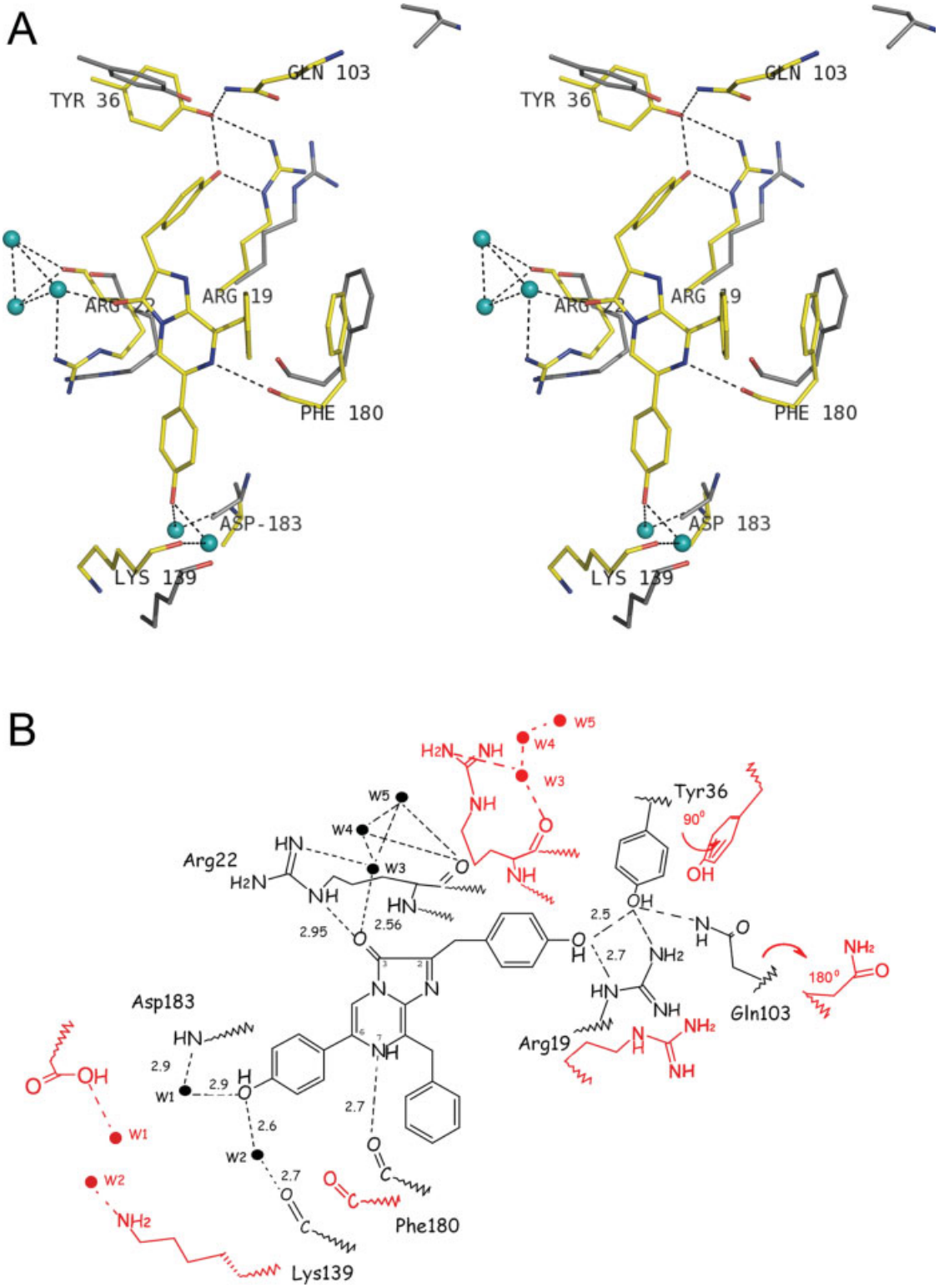

Figure 5

A. Stereoview of superimposition of key cavity residues in CBP (yellow) before and after calcium binding (gray). Water molecules in CBP are shown as cyan balls. Inferred hydrogen bonds are the dotted lines. **B.** Two-dimensional drawing of the coelenterazine-binding cavity in CBP (in black) before and after calcium addition (red lines). Distances are in Å.

boxylic side-chain of aspartate and glutamate residues and from carbonyl groups of the peptide backbone or the side-chain of asparagine, all with a bond-length to the metal ion around 2.4 Å (see Fig. 4). A seventh ligand comes from the oxygen atom of a water molecule in turn hydrogen bonded to a serine in position 9, for loops I and IV. This last hydrogen bond is absent in loop III [Fig. 4(B)]. These are typical configurations for proteins of the EF-hand super family except that the lack of a hydrogen bond between a coordinating water and the residue in position 9, is not a frequent occurrence.[24] A generalization is that proteins of this family having high affinity Ca^{2+}-binding sites, have either no water or at most one water ligand. Since the each of the three Ca^{2+}-binding sites of apo-CBP contain only one water molecule, they all should have a high affinity for the Ca^{2+}. However, only two high affinity Ca^{2+}-binding sites were observed by titration experiments for native CBP (LBP) of *R. reniformis*.[3] These comparisons show that Ca^{2+}-binding loop I undergoes the largest change in conformation, then loop IV, and those residues forming loop III hardly shift on the Ca^{2+} ligation, perhaps the explanation for the lack of interaction in loop III between the Ser120 and the coordinating water.

Coelenterazine-binding cavity

Figure 5(A) is a magnified view of the cavity part of the structure and shows that several residues adjacent to loop I are also repositioned as a result of the Ca^{2+} binding there. Some of these comprise the binding cavity and interact directly with the bound coelenterazine in CBP.[8] Interestingly, the Arg19-Tyr36 forms a "lid" over the coelenterazine-binding cavity, which becomes opened up in the Ca^{2+}-loaded structure.

Figure 5(B) is a two dimensional picture to show more clearly the residue side-chain interactions that bind the coelenterazine in the CBP cavity and the shifts induced by Ca^{2+} binding. In addition to these shifts there is a slight decrease in cavity volume, 1044–960 $Å^3$, and a change of shape. The C-terminus part of the cavity caves in, the N-terminus domain is slightly opened up and helices C, D and E move opposite each other to form a new solvent exposed broad area (see Fig. 3). The mechanism by which the coelenterazine dissociates becomes clear from this 2D diagram and is supported by structural modeling using appropriate software and the available PDB files. The strongly interacting residues in the CBP cavity are seen to have moved away such that one cannot place a model structure in any position so that the original hydrogen bonds, specifically those to the waters, W_1-W_5, can reform. Thus, the interactions are weakened and the coelenterazine escapes through the opened Arg19 lid and "hole" formed by helices C, D, and E.

The conformational changes around the cavity can be examined in detail from the PDB files. The Arg22 side chain bends and moves away along with its three water molecules, from its original position that formed a cap over the coelenterazine C3-oxygen. Interactions at the original 2-(*p*-hydroxybenzyl) of coelenterazine are lost. The Tyr36 benzyl ring is rotated 90°, the Gln103 side chain is turned over 180°, and the Arg19 side chain is moved slightly up towards the outside of the cavity [Fig. 5(B)]. Lys139 and Asp183, which indirectly coordinated the 6-(*p*-hydroxyphenyl) of coelenterazine via W_1 and W_2 come close together but still remain H-bonded to the two water molecules, although with some shift of the H-bond donor atom. Last but not least in significance, is the shift of Phe180, as this interaction was proposed in our previous article[8] as affecting the pK of this N(7) thus inhibiting formation of the anion species there, the one required for the oxygen addition reaction. In addition to these residues, which directly participated in coelenterazine binding, there are major changes in a pool of hydrophobic side chains like Phe, Met, Val, Leu, that were contributing hydrophobicity in the CBP cavity. These Ca^{2+}-induced conformational shifts taken together, simply explain the loss of affinity of the cavity for coelenterazine, whereby this substrate becomes readily available for the bioluminescence reaction with luciferase.

Table IV
Molecular Docking Computation[a] of *Renilla* luciferase to the Ca^{2+}-Loaded apo-CBP

Solution No.	Score	Area	ACE
1	13,140	1665.80	213.93
2	12,100	1557.60	374.56
3	11,916	1617.00	93.12
4	11,688	1614.50	104.40
5	11,658	1470.30	376.48
6	11,626	1461.90	388.49
7	11,480	1492.00	350.31
8	11,390	1551.20	249.01
9	11,294	1361.20	327.86
10	11,284	1494.30	25.71
15	10,942	1386.30	−58.37
16	10,874	1719.20	494.76
17	10,836	1528.30	438.88
18	10,804	1555.50	456.17
19	10,784	1361.20	416.36
20	10,732	1685.90	449.93

The top 20 solutions based on the shape complementary principle are listed. The first columun is the geometric shape complementarity score, the next is the area of interaction, and ACE is the desolvation energy.
[a]Ref. 23.

Docking computation

Table IV is an abbreviation of the PatchDoc output file, listing the top 20 docking solutions for a putative *Renilla* luciferase complex with Ca^{2+}-loaded apo-CBP. They are arranged by geometric shape complementarity score and the first solution in a row with highest score

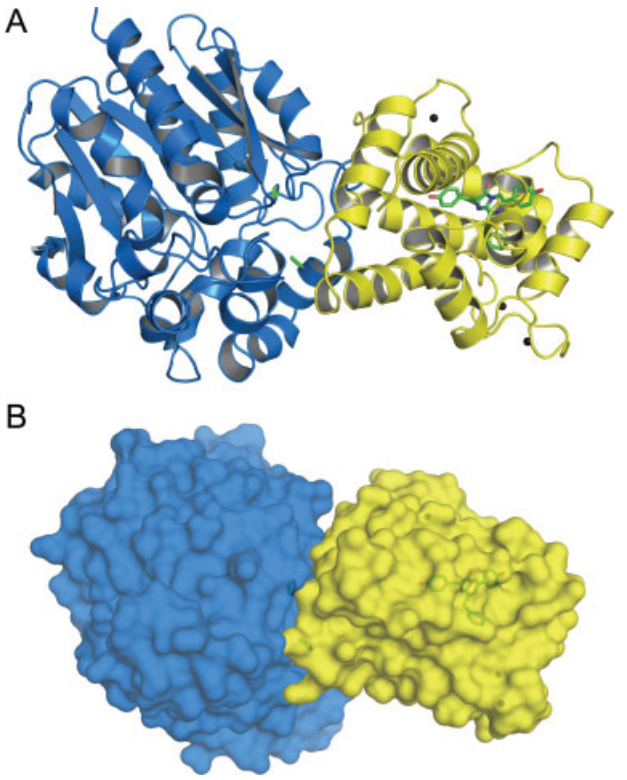

Figure 6

Computational docking of *R. muelleri* luciferase (blue) to the Ca^{2+}-loaded apo-CBP (yellow). **A** and **B** are the ribbon and molecular surface representations of the computational solution 1 from Table IV. The coelenterazine molecule is not actually present in the apo-CBP structure, but is placed there for the purpose of superimposing the CBP and apo-CBP structures. Black balls show calcium ions in apo-CBP; green pentagons show the two imidazole molecules observed in the *Renilla* luciferase structure (PDB 2PSD).

and largest interface area of interaction of the two proteins, is displayed in Fig. 6.

The authors of PatchDoc point out that a "near-native result is found among the top 100, and very often among the top 10 solutions" so it is interesting to find here that the model in Figure 6 places the "opened hole" in Ca^{2+}-apo-CBP, formed by helices C, D, and E and through which we propose the coelenterazine might escape, opposite the *Renilla* luciferase active site, the location of which has to be inferred from the position of two imidazole molecules that are known inhibitors.[22] Allowing caution in interpreting this computational solution, at least the result gives some credence to the idea that the bioluminescent reaction might occur in a luciferase-CBP complex.

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