Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution

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Calcium ATPase is a member of the P-type ATPases that transport ions across the membrane against a concentration gradient. Here we have solved the crystal structure of the calcium ATPase of skeletal muscle sarcoplasmic reticulum (SERCA1a) at 2.6 Å resolution with two calcium ions bound in the transmembrane domain, which comprises ten α-helices. The two calcium ions are located side by side and are surrounded by four transmembrane helices, two of which are unwound for efficient coordination geometry. The cytoplasmic region consists of three well separated domains, with the phosphorylation site in the central catalytic domain and the adenosine-binding site on another domain. The phosphorylation domain has the same fold as haloacid dehalogenase. Comparison with a low-resolution electron density map of the enzyme in the absence of calcium and with biochemical data suggests that large domain movements take place during active transport.

The calcium pump of sarcoplasmic reticulum was first identified in the ‘relaxing factor’ of muscle contraction and gave rise to the calcium theory that Ca²⁺ is a fundamental and ubiquitous factor in the regulation of intracellular processes. It is an integral membrane protein of relative molecular mass 110,000 (M, 110K) and pumps Ca²⁺ released in muscle cells during contraction back into the sarcoplasmic reticulum using the chemical energy of ATP, thereby relaxing muscle cells. This pump is therefore an ATPase and a representative member of a group of enzymes called P-type ATPases; the name came from the fact that they are autophosphorylated (at Asp 351 with Ca²⁺-ATPase) during the reaction cycle (for recent reviews, see refs 2–4). P-type ATPases are all ion pumps of crucial importance, for example Na⁺K⁺-ATPase and gastric H⁺K⁺-ATPase.

The amino-acid sequences of P-type ATPases have been known for some time, and secondary-structure prediction has proposed models with ten transmembrane helices (M1–M10). Extensive mutational studies have been carried out to identify the amino-acid residues that are critical for ion transport (for example, see ref. 3). Negatively charged residues on four putative transmembrane helices (M4–M6 and M8) have been postulated to form high-affinity binding sites. The evolutionary connections with other enzyme families have been a long-standing issue, because P-type ATPases lack the P-loop commonly found in other ATPases and GTPases. Nevertheless, marked homology with L-2-haloacid dehalogenase has been pointed out for the catalytic core of the cytoplasmic domain.

Direct structural information has so far been limited because the crystals that had been obtained were useful only for electron microscopy. Two types of crystal have been obtained for sarcoplasmic reticulum Ca²⁺-ATPase: tubular crystals formed in the absence of Ca²⁺ and the presence of decavanadate; and three-dimensional microcrystals formed in the presence of millimolar Ca²⁺ (refs 10, 11). From electron microscopy of these crystals, it is clear that the enzyme has a large cytoplasmic ‘headpiece’ connected to the transmembrane region by a short stalk segment and that the headpiece appears to split on Ca²⁺ binding to the membrane domain.

Here we have improved the crystallization conditions and succeeded in growing crystals suitable for X-ray crystallography. We describe the structure of the sarcoplasmic reticulum calcium pump with two Ca²⁺ ions in the transmembrane binding sites at 2.6 Å resolution. The atomic model explains the 8 Å resolution map obtained from tubular crystals, suggesting that large concerted domain movements take place during active transport.

Figure 1 Crystal packing of Ca²⁺-ATPase in the plate-like crystals. Cα traces with the a axis horizontal and the b axis normal to the plane of the paper. Thin lines represent the molecules offset by a half unit cell along the b axis owing to the crystal symmetry. Because the crystal is made of stacks of membranes, the lipid bilayers extend parallel to the ab plane (normal to the plane of the paper) but may well be distorted around the protein molecules. Horizontal bars show rough estimates of the positions of the membrane surface from the distribution of hydration water. Three cytoplasmic domains (A, N and P) are identified as in Fig. 2. Locations of two Ca²⁺ ions are indicated by spheres in violet in the transmembrane domain (M). Those of lanthanides (La³⁺ and Tb³⁺) in derivative crystals (see Table 1) are marked by circles. No binding of lanthanides was detected in the transmembrane region. The distance between adjacent layers measures 145.7 Å.
Table 1 Summary of crystallographic analysis

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<th>Data set</th>
<th>Concentration (mM)</th>
<th>Resolution (Å)</th>
<th>Completeness (%)</th>
<th>Rmerge † (%)</th>
<th>Redundancy</th>
<th>I₀/Ij</th>
<th>Riso § (%)</th>
<th>No. of sites</th>
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Reefinement statistics

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<th>No. of water molecules</th>
<th>No. of Ca²⁺ atoms</th>
<th>Rcryst (%)</th>
<th>Rfree ¶ (%)</th>
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* Diffraction data were collected at SPring-8 (beamline BL41XU (λ = 0.800 Å) and BL44B2 (λ = 0.890 Å and 1.000 Å) for native crystals and BL44B2 (λ = 0.890 Å) for derivatives).
† Rmerge = Σhkl \( |I_hkl| - \frac{\sum_{i} I_{i_hkl}}{|hkl|} \) / \( \sum_{i} I_{i_hkl} \).
‡ Riso = \( \frac{\sum_{hkl} |F_{obs}(hkl)| - |F_{calc}(hkl)|}{\sum_{hkl} |F_{obs}(hkl)|} \).
§ Defined as the ratio of the r.m.s. value of the heavy atom structure factor amplitudes and the r.m.s. value of the lack-of-closure error.
¶ Same as Rcryst, but calculated with 10% of data set aside for refinement.
# The numbers in the parentheses refer to those in the last resolution shell (2.68±2.60 Å for the native data set).

Figure 2 Architecture of the sarcoplasmic reticulum Ca²⁺-ATPase. a-Helices are represented by cylinders and b-strands by arrows, as recognized by DSSP. Cylinders are not used for one-turn helices. Colour changes gradually from the N terminus (blue) to the C terminus (red). Three cytoplasmic domains are labelled (A, N and P). Transmembrane helices (M1–M10) and those in domains A and P are numbered. The model is orientated so that transmembrane helix M5 is parallel to the plane of the paper. The model in the right panel is rotated by 50° around M5. The M5 helix is 60 Å long and serves as a scale. Several key residues are shown in ball-and-stick, and TNP-AMP by CPK. D351 is the residue of phosphorylation. Two purple spheres represent Ca²⁺ in the transmembrane binding sites. The binding sites for phospholamban (PLN) and thapsigargin (TG) are marked, as are major digestion sites for trypsin (T1 and T2) and proteinase K (PrtK). The arrow specifies the direction of view in Fig. 6b. Figure prepared with MOLSCRIPT. © 2000 Macmillan Magazines Ltd
Structure determination

The crystals were grown at pH 6.1 in the presence of 10 mM CaCl₂ and exogenous lipid. Electron microscopy of these crystals showed that the protein molecules were indeed embedded in lipid bilayers (data not shown). They were very thin (typically < 20 μm); but each of them allowed a full data set to be collected at SPring-8. The structure was solved by standard multiple isomorphous replacement (MIRAS) methods and refined to 2.6 Å resolution. The quality of the diffraction data and the refinement statistics are given in Table 1. The atomic model included about 250 water molecules in the cytoplasmic and the lumenal regions and about 30 in the transmembrane region. The distribution of hydration water molecules indicated approximately the position of the membrane boundary, which was roughly parallel to the α axis (Fig. 1).

Architecture of the sarcoplasmic reticulum Ca²⁺-ATPase

The overall architecture of the sarcoplasmic reticulum Ca²⁺-ATPase with bound Ca²⁺ is shown in Fig. 2. All 994 amino acid residues were identified in the electron-density maps (see Fig. 3a, b for the initial maps). The molecule fits in a box of 100 Å × 80 Å × 140 Å. The cytoplasmic headpiece has a split appearance consisting of three well separated domains (designated as A, N and P). The phosphorylation site, Asp 351, is located in domain P, and, as described below, the adenosine moiety of nucleotide (here we used 2',3'-O-(2,4,6-trinitrophenyl)-AMP (TNP-AMP)) is bound to domain N.

The transmembrane region (M) comprises ten α-helices (M1–M10), the arrangement of which is shown in Fig. 3a. There is a clear segregation between M1–M6 and M7–M10, consistent with the lack of M7–M10 helices in bacterial type I P-type ATPases (for example, ref. 2). The lengths of helices and the inclination to the membrane vary substantially (Fig. 2). Some of them (M2 and M5) are very long (~60 Å) and fairly straight, but some are unwound (M4 and M6) or kinked (M10) at the middle of the membrane. The helices that have been presumed to form Ca²⁺-binding sites (M4–6, M8) show very different folding patterns, although similarity among them was expected from mutation studies. M5 is located in the centre of the molecule and extends from the luminal surface of the membrane to the centre of the cytosolic domain P, looking like the 'centre mast' of the enzyme. M2 and M3 are long helices that are rather isolated. The implication of this feature is discussed below. These long helices are continuous across the membrane surface and therefore treated as single helices, including the region previously assigned to 'stalk helices'. Lumenal loops are short except for the one connecting M7 and M8 (L78, ~35 residues). In

![Figure 3](https://example.com/figure3.png)

Figure 3 The arrangement of transmembrane helices (a), an enlarged view of a part of M5 helix (b) and water accessible cavities on the cytoplasmic surface (in stereo, c). The viewing direction is roughly normal to the membrane from the cytoplasmic side with domain A on the right hand side (a,c). The luminal half including the Ca²⁺-binding sites is shown in a and the cytoplasmic half in c. Cyan spheres represent two Ca²⁺ ions (I and II) and red spheres represent water molecules. A solvent flattened map is used in a and b (calculated at 2.8 Å resolution and contoured at 1.2σ), and a 2Fₒ – Fc composite omit map prepared with CNS (calculated at 2.6 Å resolution and contoured at 1.5σ) in c. The arrow in a indicates the viewing direction in Fig. 4b. Circle in a indicates a likely position of the outlet of Ca²⁺ pathway. The water molecule found nearest to the Ca²⁺-binding sites is marked (asterisk in c). Note that only M2, M3 and M5 are labelled in c.
Fig. 2, the major sites for protease digestion and the binding sites of phospholamban and thapsigargin are also identified.

**Structure of the Ca\(^{2+}\)-binding sites**
The M4–M6 and M8 helices surround two high-density peaks (Fig. 4), which we identify as two bound Ca\(^{2+}\) ions and not water. Our reasons are fourfold. (1) The level of density is too high to be explained by water—the temperature factor was refined to unreasonably low values (<2 Å\(^2\)) when water molecules were assigned but was appropriate when Ca\(^{2+}\) were assigned (~40 Å\(^2\), similar to those of coordinating atoms). (2) At least six coordinating oxygen atoms are located at a distance of 2.2–2.6 Å from the centre of each site; this is unusual for a water molecule. (3) The valence values calculated from the geometry of coordinating atoms are 1.95 (I) and 2.15 (II), which qualify as high-affinity Ca\(^{2+}\)-binding sites. (4) Omit difference maps showed the presence of a much smaller peak 2.4 Å away from the main one (Fig. 4, I). This peak had a density appropriate to a water molecule, as judged by the refined temperature factor.

These two sites are at similar heights with respect to the membrane (Fig. 1) and are 5.7 Å apart (Fig. 4). Following previous proposals, they are termed as sites I and II. Site I is located in the space between the M5 and M6 helices with contribution from M8 at a rather distal position. The side-chain oxygen atoms of Asn 768, Glu 771 (M5), Thr 799, Asp 800 (M6) and Glu 908 (M8) contribute to this site, in agreement with mutational studies. Disruption of helix conformation around Asp 800 and Gly 801 allows both Thr 799 and Asp 800 on M6 to contribute. All of the side-chain oxygen atoms, except for that of Glu 771, are arranged on roughly the same plane; that of Glu 771 coordinates from underneath (that is, the lumenal side of) the plane (Fig. 4b). In contrast, site II is formed almost ‘on’ helix M4 (Fig. 3a) by the main-chain carbonyl oxygen atoms of Val 304, Ala 305 and Ile 307 (M4) and the side-chain oxygen atoms of Asn 796, Asp 800 (M6) and Glu 309 (M4). Obviously, this kind of coordination geometry is only possible because helix M4 is unwound between Ile 307 and Gly 310. The PEGL motif in this unwound region has been recognized as a key motif; the glutamate residue is replaced by cysteine or histidine in ATPases that transport heavy metals.

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**Figure 4** Details of the transmembrane Ca\(^{2+}\)-binding sites. The refined model is superimposed with a 2\(|F_o| - |F_c|\) composite-omit map prepared with CNS (blue meshes, contoured at 1.5σ). The meshes in pink show \(|F_o| - |F_c|\) map for Ca\(^{2+}\) and a bound water (cut-off at 3σ). Blue spheres represent Ca\(^{2+}\), and red spheres water molecules. Viewed roughly normal to the membrane from the cytoplasmic side (a) and parallel to the membrane (b) in stereo. The coordinations of oxygen atoms to Ca\(^{2+}\) are indicated by white dotted lines, and possible hydrogen bonds stabilizing the coordination geometry by green dashed lines. The viewing direction in b is also specified in Fig. 3a. Water molecule nearest to the Ca\(^{2+}\)-binding site is marked (asterisk). Note the unwinding of helices M4 and M6 and rows of exposed carbonyl oxygen atoms along these helices in b.
These two sites are stabilized by hydrogen-bond networks between the coordinating residues (for example, between Ala 306 and Asn 768) and between residues on other helices (for example, between Val 304 and Glu 309 on M4 and Glu 58 on M1, see Fig. 4). These hydrogen-bond networks must be important for the cooperative binding of two Ca$^{2+}$ ions (ref. 19). As Asp 800 is coordinated to both Ca$^{2+}$ atoms, the correct positioning of this residue must be very important. Furthermore, this residue is located in the unwound region between two helices (that is, the luminal and cytoplasmic halves of M6) and the carbonyl oxygen atom is highly exposed (Fig. 4b), implying that the position of this key residue is under the control of other residues. This unwound conformation agrees well with an NMR study of isolated M6 polypeptide in detergent micelle$^{20}$.

An important question is: how do Ca$^{2+}$ ions reach the transmembrane binding sites? Owing to the lack of large vestibules similar to those found in ion channels$^{21}$, our structure does not provide a definite answer. One candidate for Ca$^{2+}$ entry is the area surrounded by M2, M4 and M6 (Fig. 3c). It is a cavity with a rather wide opening and is clearly water accessible (seven water molecules are seen in Fig. 3c). In the upper part of this cavity are located Glu 108 and Asn 111, a critical residue for ATPase activity$^{22}$. Particularly interesting are the rows of exposed oxygen atoms formed by the unwound part of M4 (Pro 312 to Glu 309) and of M6 (Gly 801 and Asp 800). These rows of main-chain carbonyl oxygen atoms point towards the cytoplasm (Fig. 4b), providing a hydrophilic pathway leading to the Ca$^{2+}$-binding sites. The rows constrict near the Ca$^{2+}$-binding sites, trapping a water molecule (Figs 3c and 4b, asterisk); here, the oxygen atoms are arranged so that nearly ideal hydrogen-bonding geometry is provided for this water molecule. Such a geometry must be required for displacing water molecules$^{21}$ that are bound to Ca$^{2+}$ much more tightly than to monovalent cations. The outlet of Ca$^{2+}$ is likely to be located in the area surrounded by M3–M5 (Fig. 3a, circle); here too, a ring of oxygen atoms with bound water molecules is formed.

Thus, the unwinding of transmembrane helices appears to be required for two purposes: first, to realize efficient coordination geometry; and second, to provide rows of oxygen atoms for guiding Ca$^{2+}$ to the binding site and removing water molecules at the same time.

**Figure 5** Architecture of the phosphorylation domain (P in Fig. 2) of Ca$^{2+}$-ATPase (a) and the catalytic core domain of L-2-haloacid dehalogenase (HAD) (b). Critical amino-acid residues found in HAD are all preserved and indicated in a. D351 is the phosphorylation site in Ca$^{2+}$-ATPase (a), corresponding to D10 in HAD (b). Three residues (Thr 625 to Asp 627) forming the loop connecting β-strand 2 and α-helix 3 are all critical for ATPase activity$^{26}$. Lys 684 is labelled by ATP pyridoxal$^{27}$ and Asp 703 and Asp 707 are labelled by GTPATP with Na$^{+}$/K$^{-}$/ATPase$^{46}$. Colour coding is as in Fig. 2. Numbering of the secondary structures in b is made consistent with a and different from the original$^{23}$. Red dashed lines show possible hydrogen bonds. Figure prepared with MOLSCRIPT$^{47}$.
and other ATP-derivatives is located in a short loop connecting the β-strands (Lys 492 is marked in Fig. 2; also see Fig. 7b for a top view). This binding pocket is positively charged, in marked contrast to the region around the phosphorylation site (Fig. 6b).

Domain A is the smallest of the three domains and has $M_r \approx 16$K. It comprises roughly the 110 residues between M2 and M3 (previously called the transduction domain or β-strand domain), which form a distorted jelly roll structure, and the 40 residues of the N-terminal part of the enzyme, which form two short helices. This domain is connected to the transmembrane region by long loops and is nearly isolated. The susceptibility of the loops to proteinase K attack is affected by Ca$^{2+}$ (ref. 28). The well conserved TGES motif (starting at Thr 181) is located at an edge of this domain (E183 is shown in Fig. 2). Arg 198, trypsin digestion at which is enhanced by Ca$^{2+}$ and blocked by phosphorylation, is located at the outermost loop (T2 in Fig. 2). All these biochemical data suggest that this domain moves substantially during active transport.

**Expected structure in the absence of Ca$^{2+}$**

As mentioned above, the cytoplasmic headpiece has a compact appearance in the maps derived from tubular crystals. The most likely reason for this difference from the present structure is the crystallization conditions: the tubular crystals are formed in the presence of 10 mM Ca$^{2+}$ and blocked by phosphorylation, whereas in the present structure Ca$^{2+}$ is located at the outermost loop (T2 in Fig. 2). All these biochemical data suggest that this domain moves substantially during active transport.

The fitting of these three cytoplasmic domains leaves one spherical lobe of high density (Fig. 7a, large sphere) unidentified. Its density and size indicate that it is probably decavanadate ($V_{10}O_{28}^{6-}$). This lobe is located at the side of the highly positively charged groove formed by domains N and P (Fig. 6b). The groove is narrower than in the present structure (by ~20°) and decavanadate fits in the lobe (Fig. 7a), like a ball in jaws preventing the jaws from closing. Arg 489, Lys 492 (in the red loop in domain N in Fig. 7) and Arg 678 (see Fig. 6b) are expected to participate in the binding of this large anion. This location of decavanadate will explain its inhibitory effect on ATPase activity.

To realize this arrangement, all the cytoplasmic domains have to move with respect to the M4 and M5 helices, which do not fit the density map in Fig. 7 and need to be inclined by ~20°. Domain A undergoes the largest movement of the three (nearly a 90° rotation). The TGES loop starting at Thr 181 (Fig. 7, red loop in domain A) meets the TGD motif starting at Thr 625 (Fig. 7, red loop in domain P; see also Fig. 5a). The critical importance of these two motifs has been well documented. The counterpart of Thr 625 in HAD is Ser 118 (Fig. 5b), which is thought to be the binding residue of the substrate carboxyl group. The gathering of these loops in the E2 or E3 state has been demonstrated with Na$^+$/K$^+$-ATPase.

The large movement of domain A requires changes in the orientations of the M1–M3 helices, consistent with the changes in sensitivity to proteases as mentioned previously. Domain N has changed its orientation by ~20° with respect to domain P, resulting in a similar orientation—when projected along the $b$ axis—to that observed with three-dimensional microcrystals by electron microscopy. Despite the poor agreement of helices M4 and M5, the loop connecting helices M6 and M7 (L67) fits well with the density map (Fig. 7a), indicating that it follows domain P. But its position with respect to the membrane helices is different, being much higher above the membrane surface than in the presence of Ca$^{2+}$. This feature suggests that L67 is important for concerted domain motions.

**Role of the loop connecting helices M6 and M7**

This loop (L67) runs along the bottom of domain P and in a position that can mediate interactions of domain P and the transmembrane domain (Fig. 8). The P2 helix will link the movements of M5 and the DPPR region, and the P1 helix will couple those of M4 and the central β-strand on which Asp 351 is located (also see Fig. 5a). Thus, this loop interacts directly or indirectly with most of the key parts of the enzyme. If domain P moves or the upper...
part of helix M5 or M4 moves, as suggested by the comparison with the map from tubular crystals, the movement will be transmitted to M3 and M6. Because L67 is directly connected to M6, changes induced in M6 by the binding of Ca\(^{2+}\) will be transmitted to domain P through L67 and the P1 or P2 helix.

The comparison with the map from tubular crystals also suggests a conformation change in L67. In this regard, the results of scanning cysteine mutagenesis are interesting. The pair of residues that formed disulphide bridges in the absence of Ca\(^{2+}\) were compatible with our atomic model for the luminal half but not for the cytoplasmic half of helix M6\(^{32}\). These results indicate that the cytoplasmic half of M6 may undergo rotation or winding/unwinding during Ca\(^{2+}\)-transport in concert with the movement of L67. In fact, the geometry of this region is far from an ideal helix and is not recognized as such, depending on the classification program.

Thus, it is likely that this loop is important in coordinating different parts of the enzyme. In this way it may affect Ca\(^{2+}\) binding and ATP hydrolysis\(^{35}\). Asp 813 and Asp 818 were postulated to be the initial Ca\(^{2+}\)-binding sites\(^{38}\), but in this configuration they are separated by ~10 Å (Fig. 2) and are unable to form a Ca\(^{2+}\)-binding site.

**Large domain motion during ATP-hydrolysis**

The phosphorylation site (Asp 351) is more than 25 Å away from the bound nucleotide (Fig. 6b). This means that domain closure must occur during ATP hydrolysis. Also, the two residues crosslinked by glutaraldehyde (Lys 492 and Arg 678; ref. 34) are separated by more than 25 Å (Fig. 6b). ATP pyridoxal labels both Lys 492 and Lys 684 in the absence of Ca\(^{2+}\) (ref. 36). All these data indicate that domain N is the occluded state by chromium-ATP. Stabilization of the ‘occluded’ state by chromium-ATP\(^{37}\) is likely to be a result of crosslinking of domains N and P by chromium-ATP.

Large domain motions require the presence of a hinge region. Domain N can be isolated by several proteases\(^{31}\), suggesting that the region around Ser 350 and Gly 609 is indeed flexible. In fact, the connection between domains N and P appears to be extremely tenuous (Figs 2 and 6b), with a well conserved Gly 354 and the invariant DPPR motif\(^{12}\) (Fig. 5a). Furthermore, the hydrogen-bond network involving Gly 354, Arg 604 and Asp 737 to Asn 739 seems to link the movement of this hinge region to that of M5, thereby effectively transmitting the phosphorylation signal to the Ca\(^{2+}\)-binding sites.

How does Ca\(^{2+}\) binding to the high-affinity sites induce large domain motions? It is tempting to postulate that domain A works as an actuator or an anchor for the movement of domain N. As already described, the susceptibility to proteases of the loops connecting domain A to the transmembrane helices changes depending on Ca\(^{2+}\) (ref. 28, 29), consistent with the idea that Ca\(^{2+}\) binding releases domain A. This movement must be made by helices M1–M3, which have only a few hydrogen bonds with other parts of the enzyme. For example, M3 is hydrogen-bonded to L67 near the cytoplasmic end (Fig. 8) and only with M4 at either end. Exactly the same applies to M2. M1 has hydrogen bonds with M4 only, and the residues involved are Glu 309 (Fig. 4) and Lys 297, which are both critical residues\(^{6}\). Therefore, Ca\(^{2+}\) binding probably stabilizes these hydrogen bonds while destabilizing others, and moves M1–M3 to release domain A. In this regard, it is particularly interesting that thapsigargin, a very potent inhibitor\(^{38}\), is supposed to bind to the region where M3, M4 and L67 gather (around Phe 256; ref. 17; Fig. 8). This must be a very strategic point for fixing them in one configuration.

As already noted, L67 may work as a linker between different domains to elicit large-scale domain motions for transporting ions against a concentration gradient.

**Conclusion**

We have described the atomic model of Ca\(^{2+}\)-ATPase with bound...
Ca\textsuperscript{2+} on the basis of X-ray diffraction data at 2.6 Å resolution. Our model explains many more experimental results than we can describe here. We have shown that the cytoplasmic region consists of three well separated domains and have proposed new names for them: domain A may work as an actuator or an anchor for domain N, which binds nucleotides; and domain P contains Asp 351, the binding site(s)\textsuperscript{4}. We also have described how two Ca\textsuperscript{2+} ions are different domains will settle much of the controversy on the ATP-dependent ATPase. We have fitted the atomic model to a low-resolution map by electron microscopy was first done manually using Turbo-Frodo and then refined by calculating the correlation function with magnification correction of the electron microscopy map.

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