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A structural rearrangement of the Na⁺/K⁺-ATPase traps ouabain within the external ion permeation pathway

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Abstract

Using the energy of ATP hydrolysis, the Na⁺/K⁺-ATPase is able to transport across the cell membrane Na⁺ and K⁺ against their electrochemical gradients. The enzyme is strongly inhibited by ouabain and its derivatives, some that are therapeutically used for patients with heart failure (cardiotonic steroids). Using lanthanide resonance energy transfer (LRET), we trace here the conformational changes occurring on the external side of functional Na⁺/K⁺-ATPases induced by the binding of ouabain. Changes in donor/acceptor pair distances are mainly observed within the α subunit of the enzyme. To derive a structural model matching the experimental LRET distances measured with bound ouabain, molecular dynamic simulations were carried out with energy restraints applied simultaneously using a novel methodology with multiple noninteracting fragments. The restrained simulation, initiated from the X-ray structure of the E₂(2K⁺) state, became strikingly similar to the X-ray structure of the sodium-bound state. The final model shows that ouabain is trapped within the external ion permeation pathway of the pump.

Graphical abstract

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Author Contributions

FB, BR and MH conceived and supervised the project. JESR performed the LRET experiments. PM designed and obtained pump constructs and participated in some experiments. FKA and BR designed and performed molecular dynamic simulations. All authors contributed to writing the manuscript.

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Introduction

Since its discovery [1], the Na^+/K^+ -ATPase has been extensively studied because of its importance in restoring the Na^+ and K^+ gradients in cells [2] and its clinical relevance as the site of action for cardiotonic steroids used by heart failure patients.

The Na^+/K^+ pump is a P-type ATPase that hydrolyzes one molecule of ATP to drive the transport of 3Na^+ by 2K^+ against their electrochemical gradients. To function, the Na^+/K^+ pump requires two subunits: α and β . The α subunit is about 1,000 amino acids long containing ten transmembrane α helices ($\alpha\text{M1-M10}$) with intracellular N- and C-termini. The entire transport machinery resides within the α subunit [3,4]. The β subunit (~300 amino acids) has only one transmembrane segment with a large external glycosylated C-terminus domain. It functions as a chaperone subunit with some regulatory transport properties [5–8]. A third tissue-specific player in mammals is a FXYD type subunit that spans once across the membrane with an extracellular N-terminus and it is important for enzymatic modulation [9–11].

Due to its physiological and clinical relevance [12–21], interactions of cardiotonic steroids with the Na^+/K^+ pump has been extensively studied [22–37]. Ouabain (a type of cardiotonic steroid) is frequently used as the inhibitor of the Na^+/K^+ pump in ligand-receptor studies. Ouabain accesses the Na^+/K^+ pump from the extracellular side. Functional and structural studies show that ouabain binds to E_2P states containing no ions, or loaded with Na^+ , K^+ or even Mg^{2+} [31,35–37]. Ouabain's site of action is in the transmembrane region of the α subunit [31,34,36,37]. In particular, it appears to sit deep within the ion permeation pathway in a cleft between transmembrane segments $\alpha\text{M1-M6}$. Crystallographic data show that ouabain binding produces substantial rearrangements within the α subunit's transmembrane segments $\alpha\text{M1-M4}$ [31,36,37].

Here we use a spectroscopic approach to determine donor/acceptor pair distances upon binding of ouabain to fully functional Na^+/K^+ -ATPases. Distances were determined by assessing lanthanide resonance energy transfer (LRET) between 11 donor/acceptor pairs on

the extracellular side of the α and β subunits. In agreement with structural data [31,36,37], ouabain binding produced significant movements of the α subunit, while the β remained unperturbed. We have used molecular dynamics simulations to model the ouabain-bound state of the Na^+/K^+ -ATPase that corresponds to the LRET distances obtained experimentally. The initial model of the Na^+/K^+ -ATPase was built based on the crystal structure of the pump in the E_2P state [4], and was simulated in the presence of additional restraints corresponding to the donor/acceptor pair distances estimated in the presence of ouabain. To impose the experimentally obtained distances, we have developed a computational strategy, in which the donor/acceptor pairs are invisible to each other. Six donor/acceptor pairs, including three LBT (lanthanide binding tag, see Material and Methods) segments and three Cys-BODIPY FL elements are inserted into the atomic model of the pump. This atomic model is then subject to molecular dynamics simulation in which experimentally measured LRET distances are imposed as harmonic restraints between donor/acceptor elements. To mimic the experimental setup, where each donor-acceptor distance is measured independently, the interactions between each donor/acceptor pair are turned off in the simulation. In this setup, the donor and acceptor elements from a single distance measurement see each other and interact with the rest of the protein, but the other donor/acceptor pairs are invisible to them. This setup allows us to simultaneously impose the six experimentally measured distances without interference of the long LBT loops.

Our results indicate that αM1 and αM2 helices opened up to accommodate the bound ouabain, as the αM3 and αM4 helices moved toward the ouabain binding site. Interestingly, the transmembrane region of the final model is closer to the crystal structure of the Na^+ bound E_1P -ADP state [38] rather than the E_2P -ouabain bound structure [37].

Results

The objective of these experiments is the direct estimation of inter and intra subunit conformational changes that occur when a functional Na^+/K^+ pump binds the inhibitor ouabain. To this end, experiments were conducted in oocytes expressing squid Na^+/K^+ pump constructs containing one donor (LBT(Tb^{3+})) and one acceptor (BODIPY FL dye). All constructs were tested to be functional and sensitive to ouabain (Figure S1). Molecular distances were estimated from donor and sensitized emission decays in the absence and in the presence of ouabain.

Inter-subunit distance determinations upon ouabain binding

We estimated α - β distances in three donor-acceptor pairs, first in the absence and then in the presence of ouabain bound. Figure 1 (A & B) shows an example in the absence of ouabain, when the donor is in position 324LBT(αM3 - M4) and the acceptor is a BODIPY FL dye attached to a cysteine located at the external end of the β subunit's transmembrane segment (T71C(β)). The donor emission (with no acceptor) shows the characteristic slow decay of ~ 2.4 ms when a Tb^{3+} is tightly bound to the LBT moiety (Figure 1A). The sensitized emission decay detected in the first dark region of the Tb^{3+} emission spectrum is much faster ($\tau=1.36$ ms; Figure 1B), indicating an energy transfer between the Tb^{3+} in the LBT and the fluorophore. Is the observed energy transfer specific to this donor-acceptor pair? To

address this question we performed experiments with pumps formed by the 324LBT(α M3–M4) and the wild-type β subunit subjected to the same experimental protocol including BODIPY FL labelling (Figure 1C & 1D). In this case, the donor emission decay is similar (Figure 1C) confirming the presence of 324LBT(α M3–M4) subunits in the membrane. However, the sensitized emission is virtually absent (Figure 1D), demonstrating that the decay observed previously is specific to the donor/acceptor pair assessed.

Next we asked whether the sensitized emission of the 324LBT(α M3–M4)/T71C(β) pair changes in the presence of ouabain in its binding site. Figure 2A shows the sensitized emission decays in the same oocyte before (black) and after incubating the oocyte with 100 μ M ouabain (gray). The presence of the inhibitor slowed down the decay from 1.34 to 1.59 ms, suggesting that the donor and the acceptor increased their distance after ouabain binding. The donor emission decay is not changed by the presence of ouabain (Figure S2), showing that the inhibitor molecule does not distort the integrity of the LBT-Tb³⁺ complex. Therefore the changes in sensitized emission with ouabain reflect unambiguous movements between the donor and the acceptor. Similar results were obtained with donor-acceptor pairs 125LBT(α M1–M2)/T71C(β) and 988LBT(α M9–M10)/T71C(β) (Figure S3). Figure 2B displays the estimated distances before and after ouabain incubation of the three inter-subunit donor-acceptor pairs. In all cases, the presence of ouabain in the pump increased the distances between α and β subunits.

Intra-subunit distance determinations upon ouabain binding

The inter-subunit distance changes observed could originate from movements of the subunits α or β , or a combination of both. To estimate intra β subunit distances we introduced a LBT moiety at position 72, close to the external end of the transmembrane segment (72LBT(β)) and mutated to cysteine several residues in different regions of the subunit. Figure 3A shows the sensitized emission decays with the pump construct α WT/72LBT(β)-N120C(β) without (black) and with ouabain bound (gray). The time constants are indistinguishable (1.13 and 1.07 ms), suggesting that the β subunit does not change upon ouabain binding. Distance estimations for this pump construct and, constructs α WT/72LBT(β)-S127C(β) and α WT/72LBT(β)-R268C(β) do not show conformational changes of the β subunit in presence of ouabain (Figure 3B). It is then likely that inter-subunit distance changes detected previously come from the α subunit.

Intra α subunit distances were estimated with 125LBT(α M1–M2), 324LBT(α M3–M4) and 988LBT(α M9–M10) as donors, and cysteines at the external end of transmembrane segments 5 through 7. Figures 4A & 4B show two examples with pump constructs 324LBT(α M3–M4)-S886C(α M7)/ β WT and 125LBT(α M1–M2)-I810C(α M6)/ β WT. In both cases, ouabain in the pump altered the sensitized emission decay. In the first case it was slowed down while in the second it was sped up. Distance estimations from these results and those obtained with three other intra α subunit pump constructs are shown in Figure 4C. Remarkably, in all cases the distance between the donor and the acceptor was changed by the presence of the ouabain molecule in the pump.

Discussion

We used a LRET-based method to study the conformational changes induced by ouabain binding to the Na⁺/K⁺-ATPase. We designed donor and acceptors sites in all external loops of the α subunit, as well as in different sites within the β subunit. We were unable to detect changes in donor-acceptor distances within the β subunit, which is compatible with crystal structures of Na⁺/K⁺-ATPase with ouabain bound [31,36,37]. In these structures, binding of ouabain produced rearrangements at the extracellular end of the transmembrane segments α M1– α M4. Similarly, our LRET determination show that ouabain binding produce distance changes between the linkers α M1–M2, α M3–M4 and α M9–M10 and regions of the β and α subunits. We have used molecular dynamics simulations to model the ouabain-bound state of the Na⁺/K⁺-ATPase that corresponds to the LRET distances obtained. The initial model of the Na⁺/K⁺-ATPase was built based on the crystal structure of the pump in the E₂P state [4]. This model was then simulated in the presence of additional restraints corresponding to the experimentally estimated distances between donor-acceptor pairs in the presence of ouabain, to give the final model that represents the ouabain bound state (see Methods). Figure 5A and 5B shows side views of the superposition of the initial and final models, with an ouabain molecule bound. The α M5–M10 regions (shown in green) remained very similar between the two models, and for clarity only that from the final model is shown. The transmembrane segment of the β subunit is represented in red. In our models, the relative position of the β subunit's transmembrane segment remained similar as observed in different ouabain bound crystal structures [31,36,37]. The moving parts upon ouabain binding were the α M1–M4 helices. Their positions in the initial model are shown in light gray and in a different color for the final model. During the simulation, α M1 and α M2 helices opened up to accommodate the bound ouabain (Figure 5C; top view). These two helices moved together and bulged in the middle; their displacement at the extracellular end of the helices is approximately 6 Å. In addition, α M3 and α M4 helices moved towards the ouabain binding site. Interestingly, the α M4 helix bends in the middle of the membrane in a hinge-like motion resulting in a displacement of approximately 7 Å at near the extracellular side. This movement of the α M4 segment is followed by the α M3 helix, which moves as a rigid body. Even though the details of the local movements predicted by modeling cannot be directly compared with known ouabain-bound crystal structures since likely represent different conformations, general principles about these motions can be drawn. First, α M1 and α M2 helices are opened up to accommodate the ouabain molecule, suggesting that initial access of ouabain originate from a state in which the external access channel is opened like in the SERCA1a E₂:BeF₃[–] structure [39]. A further movement towards the cytoplasmic end of α M1 and α M2 helices has also been observed in ouabain bound crystal structures [36,37] and functionally suggested by ouabain induced changes of trypsin accessibility in the α M2– α M3 linker [40]. Second, α M4 helix appears to be rather flexible and prompt to adopt changes in conformation upon ouabain binding as observed in our simulations and ouabain bound crystal structures [36,37]. Third, once ouabain is bound, the molecule is trapped within the ion permeation pathway unable to freely escape to the external solutions, as seen in our simulations and ouabain bound crystal structures [36,37].

Comparison of the final model with the crystal structure of the E₂ ouabain [37] (Figure 6B) and the Na⁺ bound states [38] (Figure 6C) shows that while our initial structure was very similar to the E₂ ouabain structure (Figure 6A), the final model is remarkably closer to the Na⁺ bound state (Figure 6C). Distinct differences still exist between the positions of αM1–M2 helices in the two models, which can be due to the fact that the cytoplasmic domains of the pump and in particular the A domain were constrained to their initial positions (as in E₂P) during the simulations. However, αM3 and αM4 helices have moved to the same position as in the Na⁺ bound state. The bending movement of the αM4 segment, which partially coordinates the ions in the middle of membrane, is similar to the movement observed between the Na⁺/K⁺-ATPase's crystal structures with K⁺ bound [4] and Na⁺ bound [38] states, suggesting that the LRET distances as well as the final model obtained here correspond to the Na⁺ bound high affinity binding state of ouabain [35]. These discrepancies between the crystal structures of Na⁺/K⁺-ATPase with ouabain and our model are readily explained by the fact that all models derived from crystal structures have been obtained in the absence of Na⁺, reflecting distinct conformations of ouabain bound Na⁺/K⁺-ATPases.

Materials and Methods

LRET measurements and Na⁺/K⁺ ATPase LBT constructs

We use a variant of the LRET energy transfer technique in which the donor is a Tb³⁺ bound with high affinity to a 17 amino acid long tag (YIDTNNDGWYEGDELLA) that can be inserted at any desired location within a protein[41]. The Lanthanide Binding Tag (LBT) contains a Trp residue that acts like an antenna to excite the Tb³⁺. In this study we used four LBT constructs in the squid (*Loligo opalescens*) Na⁺/K⁺-ATPase [42]. In three of them, LBT was inserted in the α subunit and in one construct the LBT was inserted in the β subunit. 125LBT(αM1–M2) denotes an α subunit LBT construct in which the LBT was inserted after position 125 located between transmembrane segments 1 and 2. Following the same nomenclature, the other two α subunit LBT constructs are 324LBT(αM3–M4) and 988LBT(αM9–M10). 72LBT(β) represents the construct in which the LBT was inserted in the β subunit at the external end of the transmembrane segment. The acceptor was a BODIPY FL fluorophore molecule attached to a cysteine mutation in the α or β subunit. In total, we studied 11 donor-acceptor pairs: 3 α-β, 5 α-α and 3 β-β. Measurements using LRET technique and its advantages have been lengthily discussed [43]. The specific details of our system and measurements have been described previously [34,44]. For each oocyte expressing a donor-acceptor pair we performed distance determinations in the absence or in the presence of ouabain bound to the Na⁺/K⁺ATPase. Oocytes were preincubated with 100 μM ouabain for 5 min before measurements were made. In all constructs ouabain inhibition is irreversible in the time frame of our experiments rendering an unperturbed high affinity binding site. The donor's emission decay was acquired in a solution containing 5 μM Tb³⁺ (TbCl₃, Sigma Aldrich) in oocytes that were not labeled with BODIPY FL dye (Invitrogen). The sensitized emissions were acquired in oocytes that were pre-incubated with BODIPY FL dye for 20 minutes. The sensitized emission decays were detected within the first dark region of the Tb³⁺ emission spectrum (500–540 nm). Distances between the donor and acceptor were estimated from the Förster relation using the time constants of the donor emission (τ_D) and the sensitized emission (τ_{SE}) as: $R=R_0 (\tau_{SE}/(\tau_D-\tau_{SE}))^{1/6}$ where R₀ is 42

Å. Pump's rearrangements induced by ouabain binding were estimated in terms of the displacement $(=R_{\text{ouab}}-R)$, where R and R_{ouab} correspond to LRET-based distances first in the absence of ouabain and subsequently in the presence of ouabain bound, respectively. Means $\pm$ SEM are given or plotted and n the number of experiments is indicated by n .

Structural modeling

The homology model of the squid (*Loligo opalescens*) Na⁺/K⁺ ATPase was generated based on the crystal structure of the Na⁺/K⁺-ATPase from shark rectal gland (PDB code: 2ZXE) using the program Modeller [45]. Missing residues in the α and β subunits of the crystallographic structure were also omitted in the squid homology model.

Figure S4 shows an overview of the modeling system. Three LBT loops were inserted at positions corresponding to 125LBT(α M1–M2), 324LBT(α M3–M4), and 988LBT(α M9–M10). The LBT coordinates were taken from the PDB code 1TJB[41]. Using the program Modeller, the coordinates of the squid homology model was combined with the LBT coordinates to produce 10 different models containing the three insertions simultaneously, from which the most favorable model was used for further simulations. Three cysteine mutations were introduced at positions T71C(β), L800C(α M5) and S886C(α M7). These cysteines were covalently attached to BODIPY FL. The Cys-BODIPY FL moiety was parameterized using the General Automated Atomic Model Parameterization (GAAMP) (<http://gaamp.lcrc.anl.gov/xsede.html>) [46].

The final model was subject to molecular dynamics simulations using NAMD [47]. During the simulations, the extracellular domain of the β subunit was removed to avoid further clashes with the inserted loops. Harmonic restraints were used to impose distance restraints between each LBT(Tb³⁺)/BODIPY FL pair, constraining them to the distances estimated experimentally after ouabain is bound. All the LBT(Tb³⁺)/BODIPY FL pairs were introduced simultaneously in the structure to make full use of all the available information from the experiments. To avoid unphysical steric clashes and to mimic the experimental conditions in which each donor/acceptor distance was determined separately, these LBT loops as well as Cys-BODIPY FL side chains were treated as non-interacting fragments with respect to each other in the simulations. According to this protocol, selected nonbonded interactions (i.e. Lennard-Jones and electrostatic interactions) between atom pairs belonging to individual LBT loops or individual BODIPY FL attachments were skipped (omitted) from the energy and force calculations during the simulations. In practice, this protocol was implemented by manipulating the non-bonded exclusion list in the protein structure file (PSF) of the system for selected pairs of atoms

The protocol allows simultaneous simulations of the LBT-inserted structure with the wild-type squid model (as shown in Figure Supplementary 4). In each simulation, the LBTs as well as its ten neighboring residues, i.e. five residues before and five residues after the insertion are replicated and superimposed onto the wild-type structure. To confine the structure of the squid to adopt only one conformation, the backbone atoms of the five residues before and after the insertion are harmonically restrained to follow the coordinates of the wild-type squid structure.

To avoid structural deformation of the LBT loops, the atomic distance between each Tb^{3+} atom and its surrounding acidic residues in the LBT structure (2 Glu and 2 Asp residues) were constrained to their crystallographic distances. In addition, the backbone of the cytoplasmic domain of the pump, as well as the TM regions within 5 Å of the cytoplasmic solution was restrained.

The parameters of ouabain were obtained using the General Automated Atomic Model Parameterization (GAAMP) (<http://gaamp.lcrcl.gov/xsede.html>) [46]. The Cys-BODIPY FL parameters were also obtained similarly by first parameterizing the iodacetamide-cystine compounds and then manually adding the BODIPY FL.

The simulation systems include the α subunit of the Na^+/K^+ ATPase as well as the transmembrane region of the β subunit (residues 39–74). The lipid bilayer and solvent were not included in the system to facilitate conformational changes of the protein. The simulations are carried out using the CHARMM36 forcefield for the proteins [48] and the newly determined parameters for ouabain and BODIPY FL attachments. Electrostatic calculations were cut off at 12 Å. The simulations were carried out at a constant temperature of 300K with a timestep of 1fs. Each simulation was run for 120ps, during which the position of LBT insertions and their adjacent transmembrane helices have stabilized toward a stable conformation. LRET distances were imposed on the system as harmonic restraints between each BODIPY-LBT(Tb^{3+}) pair with a force constant of 1.0 kcal/mol.Å. Additionally, residues 900–949 and 960–990 of the transmembrane domain were constrained to their initial position to prevent tumbling and lateral movement of the protein when additional restraints are applied. The $\alpha M9$ and $\alpha M10$ TM helices containing residues 900–949 and 960–990 do not vary among the Na^+ -bound, ouabain-bound and the K^+ -bound structures of the pump and thus, restraining their backbone to the crystallographic positions is not expected to affect the results. The simulations included ouabain at the high affinity position that was identified previously [34]. During the simulation, ouabain fluctuates around its initial position. The root mean square deviation of ouabain between its initial position and its final position is 5.2 Å, in which ouabain has moved 4 Å down toward the cytoplasmic region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. Proposed model of the ouabain bound conformation of a functional Na^+/K^+ -ATPase
2. Ouabain binding changes distances at the external end of the α subunit helices
3. A molecular model shows ouabain trapped within the ion permeation pathway
4. The ouabain bound model corresponds to a Na^+ -occluded state of the Na^+/K^+ -ATPase

Fig. 1.

LRET-based measurements of energy transfer in the Na^+/K^+ -ATPase in the absence of ouabain. (a) Donor emission of the pump construct 324LBT(α M3–M4)/T71C(β). Solid line represents an exponential fit with three time constants. The fastest two components are associated with unspecific Tb^{3+} binding. The slowest component ($\tau_D = 2.39$ ms) corresponds to the emission decay from Tb^{3+} bound to LBT. (b) Sensitized emission of the pump construct 324LBT(α M3–M4)/T71C(β). Solid line denotes an exponential fit with two components. The slowest component ($\tau_{SE} = 1.36$ ms) represents the sensitized emission of the acceptor reflecting the energy transfer between the donor and the acceptor. (c) Donor emission of the pump construct 324LBT(α M3–M4)/ β WT. The slow luminescence decay ($\tau_D = 2.36$ ms) reflects the presence of protein in the membrane. (d) Sensitized emission of the pump construct 324LBT(α M3–M4)/ β WT. In this case, only unspecific signal is observed due to the lack of acceptor in the protein.

Fig. 2.

Changes in energy transfer in the Na^+/K^+ -ATPase with ouabain bound. (a) Sensitized emissions of the pump construct 324LBT(α M3–M4)/T71C(β). Sensitized emission in the absence of ouabain decayed with a τ_{SE} of 1.34 ms (black trace). In the same oocyte, preincubation with ouabain slowed down the decay to 1.59 ms (gray trace). (b) Distance estimations. Distance estimated for constructs 125LBT(α M1–M2)/T71C(β) (n=13), 324LBT(α M3–M4)/T71C(β) (n=10) and 988LBT(α M9–M10)/T71C(β) (n=5) in the absence of ouabain (black) and in the presence of ouabain bound (gray). Upper bars show the distances caused by ouabain bound to the pump.

Fig. 3.

Intra β subunit distance changes upon ouabain binding to the Na^+/K^+ -ATPase. (a) Sensitized emissions of the pump construct $\alpha\text{WT}/72\text{LBT}(\beta)\text{-N120C}(\beta)$. Sensitized emission in the absence of ouabain decayed with a τ_{SE} of 1.13 ms (black trace) while in the presence of ouabain bound (same oocyte) it decays with a τ_{SE} of 1.07 ms (gray trace). (b) Distance estimations. Distance estimated for constructs $\alpha\text{WT}/72\text{LBT}(\beta)\text{-N120C}(\beta)$ ($n=8$), $\alpha\text{WT}/72\text{LBT}(\beta)\text{-S127C}(\beta)$ ($n=10$) and $\alpha\text{WT}/72\text{LBT}(\beta)\text{-R268C}(\beta)$ ($n=5$) without ouabain (black) and in the presence of ouabain bound (gray). Upper bars show the distances caused by the presence of ouabain in the pump.

Fig. 4.

Intra α subunit distance changes upon ouabain binding to the Na^+/K^+ -ATPase. (a) Sensitized emissions of the pump construct 324LBT(α M3–M4)-S886C(α M7)/ β WT. In the absence of ouabain, sensitized emission decayed with a τ_{SE} of 1.45 ms (black trace). Ouabain bound to these pumps slowed down the sensitized emission decay to 1.69 ms (gray trace). (b) Sensitized emissions of the pump construct 125LBT(α M1–M2)-I810C(α M6)/ β WT. In this example, ouabain binding accelerated τ_{SE} from 1.30 (black trace; absence) to 1.12 ms (gray trace; presence). (c) Distance estimations. Distance estimated for all intra α subunit constructs tested: 125LBT(α M1–M2)-L800C(α M5)/ β WT ($n=7$), 125LBT(α M1–M2)-I810C(α M6)/ β WT (15), 324LBT(α M3–M4)-S886C(α M7)/ β WT ($n=7$), 988LBT(α M9–M10)-S886C(α M7)/ β WT ($n=4$) and 988LBT(α M9–M10)-I810C(α M6)/ β WT ($n=5$). Black

bars correspond to distances without ouabain while gray bars represent distances upon ouabain binding. Upper bars show the distances produced by ouabain binding.

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Fig. 5.

Modeling the ouabain-bound state of the Na⁺/K⁺-ATPase. (a) & (b), Snapshot (side views rotated ~ 180°) of the Na⁺/K⁺ ATPase after a short simulation in which the LRET distances corresponding to the ouabain-bound conformation are imposed as harmonic restraints between LBT(Tb³⁺) and the BODIPY FL. The final conformation is shown in green (αM5–M10), yellow (αM1), orange (αM2), cyan (αM3), and blue (αM4). The β subunit is shown in red. The initial conformation of αM1–M4 segments is shown in light gray. Ouabain is shown as a wired mesh in magenta. (c) Movement of the extracellular half of the transmembrane helices αM1–M4 is shown in top view. The numbers in the parenthesis correspond to the movement of most extracellular residue of each transmembrane helix after simulating the system while enforcing the LRET distances.

Fig. 6.

Comparison of the ouabain bound model with the Na⁺-bound state and the E₂P Ouabain bound state. (a) Superposition of the Na⁺/K⁺-ATPase in the E₂P ouabain bound state (white [37]) with the initial conformation of the squid model. For clarity only M1–M4 of the squid model are shown in yellow, orange, cyan, and blue, respectively. (b) Superposition of the Na⁺/K⁺ ATPase in the E₂P ouabain bound state shown in white with the ouabain-bound conformation (αM1–αM4) obtained in the simulations after imposing the LRET distances. (c) Superposition of the Na⁺/K⁺-ATPase in the Na⁺-bound state E₁ shown in green [38] with the final model of the ouabain-bound state, for which only αM1–M4 helices are shown.