RESEARCH ARTICLE

Ga₁ inhibition mechanism of ATP-bound adenylyl cyclase type 5

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Abstract

Conversion of adenosine triphosphate (ATP) to the second messenger cyclic adenosine monophosphate (cAMP) is an essential reaction mechanism that takes place in eukaryotes, triggering a variety of signal transduction pathways. ATP conversion is catalyzed by the enzyme adenylyl cyclase (AC), which can be regulated by binding inhibitory, Gaᵢ, and stimulatory, Gaₛ, subunits. In the past twenty years, several crystal structures of AC in isolated form and complexed to Gaₛ subunits have been resolved. Nevertheless, the molecular basis of the inhibition mechanism of AC, induced by Gaᵢ, is still far from being fully understood. Here, classical molecular dynamics simulations of the isolated holocenzyme type 5 and the holocbinary complex AC₅:Gaᵢ have been analyzed to investigate the conformational impact of Gaᵢ association on ATP-bound AC₅. The results show that Gaᵢ appears to inhibit the activity of AC₅ by preventing the formation of a reactive ATP conformation.

Introduction

The nucleotide adenosine triphosphate (ATP) plays a crucial role in metabolism, not only as primary energy carrier, but also in intracellular signal transduction, acting as a substrate for the formation of the second messenger cyclic adenosine monophosphate (cAMP). cAMP is produced through deprotonation and cyclization of ATP, catalyzed by the enzyme adenylyl cyclase (AC) [1, 2]. Nine different membrane-bound isoforms of AC are known in nature, ranging from AC₁ to AC₉ [3]. Structurally, such isoforms consist of two membrane regions, M₁ and M₂, and two cytoplasmatic domains, C₁ and C₂, of which the C₁/C₂ interface harbours the enzyme’s active site. Once cAMP dissociates from the C₁/C₂ interface, the second messenger is able to transduce a signal in the cell by, for example, activating protein kinase A and affecting ion channels located in the lipid bilayer [4–6]. Apart from their primary function as signal transducers, ACs may also work as signal integrators since these enzymes can act as decision functions, determining the amount and the time of cAMP release. A typical decision function, characteristic for several AC enzymes, is the detection of co-occurring signaling...
events, also known as coincidence detection [7–13], which is known to be involved in memory and reward learning mechanisms [14–16].

cAMP regulation is governed by G-protein-coupled receptors (GPCRs). These receptors can activate inhibitory or stimulatory G proteins in the cytosol to enable or disable AC’s catalytic function. When G proteins are activated by GPCRs, their heterotrimeric structure, consisting of a α, β and γ subunit, dissociates, which results in a monomeric Ga subunit and a βγ dimer [17]. Once a Ga subunit is active, it can bind to AC isoforms via non-competitive association. Inhibitory Ga (Ga_i) can interact with AC1, AC5 and AC6 via its C1 domain while stimulatory Ga (Ga_s) can bind to all isoforms of membrane-bound AC through C2 association (Fig 1) [18–20].

Several X-ray structures have been resolved for the stimulated binary complex, AC:Ga_i [21–23]. The majority of these AC X-ray structures only include the cytoplasmatic C1 and C2 domains. Nonetheless, these structures can be used to obtain a better understanding of the stimulatory effect of Ga_i on AC5 as the cytoplasmatic domains are known to remain catalytically active even in the absence of the membrane-embedded protein regions [18, 24]. In contrast to Ga_s, no X-ray structures are available for the AC:Ga_i dimer as crystallization of the myristoylated form of Ga_i is challenging [25].

Recently, classical molecular dynamics (MD) simulations have been employed to gain insight into Ga_i’s inhibitory mechanism by simulating an AC5:Ga_i and a Ga_s:AC5:Ga_i complex in the absence of ATP [26, 27], i.e. with the apo form of AC5. The results of these studies show that the apo dimer and trimer are stable and indicate that the Ga_i-bound AC5 structure undergoes conformational changes that lead to an inhibited state of the enzyme [26]. In particular, such conformational changes, occurring in both AC5:Ga_i and Ga_s:AC5:Ga_i complexes, result in a reduced accessibility of the active site, thus decreasing the probability of substrate binding and therefore their catalytic activity. However, the presence of ATP could potentially affect Ga_i’s inhibitory function as the substrate has a stimulatory effect on the catalytic function of AC5. Frezza et al., for instance, showed that the presence of ATP in the binding site of AC5 impacts the structure and flexibility of AC5 in both the isolated and Ga_i-bound state [28]. Therefore, the conformation and activity of AC5 in the AC5:Ga_i binary complex could potentially be influenced by the presence of ATP at the C1/C2 interface.

Here, molecular dynamics simulations are presented of the ATP-bound AC5:Ga_i binary complex and the holo Ga-free AC5 protein. Conformations sampled by the protein and ATP along 3 μs of simulation have been analyzed and compared between the two simulated systems. Additionally, a second simulation of the ATP-bound AC5:Ga_i binary complex was carried out for 700 ns in order to check the reproducibility of the results presented herein. The results show that the interactions between holo AC5 and Ga_i lead to a perturbation of the AC5 binding region for the stimulatory Ga subunit, which suggests that the probability for Ga_i binding is reduced. Additionally, in contrast with the holo Ga-free AC5 protein, the conformations sampled by ATP in the holo AC5:Ga_i binary complex result in a non-reactive ATP state. These two conformational changes, occurring upon Ga_i association, are suggested to be at the basis of the inhibition mechanism of AC5 mediated by Ga_i binding.

Materials and methods

Initial structures

The crystal structure (PDB ID 1CJK) of AC(ATPαS):Ga_i was used as a template for the holo binary AC5:Ga_i complex and the holo Ga-free AC system [22]. This X-ray structure includes the catalytic AC domains, C1a and C2a, bound to an ATP analogue ATPαS (Adenosine-5’-rppalpha-thio-triphosphate), and a Ga_i subunit interacting with the catalytic domains of AC. The
template used for modelling the active rat AC5 (UniProtKB Q04400) conformation in the binary complex consists of the C1 and C2 domains (C1a from canine AC5 and C2a from rat AC2) from 1CJK [22]. The Gαi1 subunit was taken from reference [29], a modelled structure of the myristoylated Rattus norvegicus Gαi1 subunit (UniProtKB P10824) interacting with guanosine-5’-triphosphate (GTP) and a Mg2+ ion. The active myristoylated Rattus norvegicus Gαi1 is referred to as Gαi1 because only a myristoylated form of Gαi1 was used in the simulations. Myristoylation, crucial for Gαi’s inhibitory function [18, 19], is a post-translational modification of the N-terminus of Gαi1 that results in the covalent attachment of a 14-carbon
saturated fatty acid to the N-terminal glycine residue of Ga\textsubscript{11} via an amide bond. The modelled AC5 and Ga\textsubscript{11} structures were used for docking Ga\textsubscript{11} on AC5’s C1 domain to obtain the initial binary complex conformation for simulation.

The HADDOCK web server [30] was used for docking ten conformations of the active Ga\textsubscript{11} subunit to AC5’s catalytic domains in the holo form as described in references [26, 29]. The active region of Ga\textsubscript{11} was defined in HADDOCK as a large part of the alpha helical domain (residues 112-167), the switch I region (residues 175-189) and the C-terminal region of the \( \alpha \)3 helix (residues 554-561) based on results from gel filtration that show that Ga\textsubscript{11} is unable to interact with C2 and mutagenesis experiments that have confirmed that Ga\textsubscript{11}’s main interactions with AC is via the C1 domain [18]. Ten snapshots of Ga\textsubscript{11} were used for docking the Ga subunit to the catalytic domain of AC5. These snapshots were extracted at time intervals of 0.5 ns from the end of the classical MD trajectory of Ga\textsubscript{11} (around 1.9 \( \mu \)s) described in reference [29]. The same three criteria for complex selection as in reference [26] were applied: (1) the absence of overlap between the C2 domain and Ga\textsubscript{11}, (2) no overlap with the GTP binding region of Ga\textsubscript{11} and the C1 domain of AC5, and (3) presence of similar complexes in the top-ten docking results of the docking calculations performed for all ten used Ga\textsubscript{11} conformations. This last criterion increases the probability that the docked orientation of the selected complex is robust as similar AC5:Ga\textsubscript{11} complexes can be obtained using different conformations of Ga\textsubscript{11}. Besides meeting these criteria, the orientation of Ga\textsubscript{11} with respect to the membrane (absent in the simulation) was assessed to select a binary complex that would be consistent with a membrane environment. Moreover, the position of Ga\textsubscript{11} on the C1 domain was required to be similar to its position on C1 in the absence of ATP [26].

**Classical molecular dynamics simulations**

*Holo Ga-free AC5* and the *holo AC5:Ga\textsubscript{11}* complex were solvated with \( \sim 31\,000 \) and \( \sim 53\,000 \) water molecules respectively. K\textsuperscript{+} and Cl\textsuperscript{−} ions were added to the system to obtain a physiological concentration of 150 mM. An excess of K\textsuperscript{+} ions was used to neutralize the system. The force fields used for the protein and the water molecules are AMBER99SB [31] and TIP3P [32], which were employed by Gromacs 5.1.2 [33, 34] to perform the simulations. The adjusted force field parameters for the Cl\textsuperscript{−} and K\textsuperscript{+} ions were taken from Joung et al. [35]. For GTP and ATP, the force field parameters generated by Meagher et al. were used [36]. The Mg\textsuperscript{2+} ion parameters originated from Allnä et al. [37] and the parameter set for the myristoyl group was taken from reference [29]. Both the *holo Ga-free AC5* and the *holo AC5:Ga\textsubscript{11}* complex were simulated for 3 \( \mu \)s at 310 K and 1 bar using a Nosé-Hoover thermostat [38, 39] and an isotropic Parrinello-Rahman barostat [40]. A replicate of the *holo AC5:Ga\textsubscript{11}* system with different initial velocities was simulated for \( \sim 0.7\,\mu\text{s} \). Electrostatic interactions were calculated with the Ewald particle mesh method [41] with a real space cutoff of 12 \( \AA \). Bonds involving hydrogen atoms were constrained using the LINCS algorithm [42]. The integration time step was set to 2 fs. Images of both protein systems were prepared with Pymol [43].

**Results and discussion**

**Stability and conformational analysis**

In order to shed light on the molecular basis of the Ga\textsubscript{11} inhibition mechanism, the *holo Ga-free AC5* protein and the *holo AC5:Ga\textsubscript{11}* complex have been simulated for 3 \( \mu \)s. The root-mean-square deviation (RMSD) of both systems (Fig 2) shows that the subunits in each complex are stable, yet, C1 domain converges between 0.15-0.20 nm, while C2 domain stabilizes...
around 0.30 nm. However, the stability of the secondary structures of C1 and C2 (see S1 and S2 Figs) and the stable number of hydrogen bonds (Hbonds) in the catalytic domains (S3 Fig) all indicate subunit stability.

Concerning the RMSD of the overall complex, holo Gα-free AC5 appears to deviate significantly less from its initial structure than holo AC5:Gαi (S4 Fig). This difference is to be expected as the initial AC5 conformation in both systems originates from an AC:Gαs structure in the absence of Gαi. Hence, in AC5:Gαi, AC5 is affected by Gαs removal as well as Gαi association, inducing a larger perturbation on the AC5 protein than in the Gα-free AC5 condition. However, the decreased radius of gyration (Rg) of the binary complex (Fig 2) implies that

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**Fig 2.** Structural stability of holo Gα-free AC5 and holo AC5:Gαi complex. Top panel: the RMSD, calculated on the protein backbone of each protein subunit with respect to the starting structure, is reported for holo Gα-free AC5 and the holo AC5:Gαi binary complex as function of time. Bottom panel: Time evolution of the radius of gyration of holo Gα-free AC5 and holo AC5:Gαi binary complex.

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interdomain interactions enhance during simulations. Indeed, the number of hydrogen bonds between AC5 and G\textsubscript{\alpha i} in the binary complex was found to increase along the simulation, which is consistent with the decrease in Rg, indicating an overall stabilization of the complex. Hence, these results suggest that the difference in overall RMSD could be due to an alteration in conformation, leading to a change in intermolecular interactions with respect to the starting structure with a possible reorientation of the system’s subunits. Such a reorientation is shown by comparing the first and the last frame of the holo AC5:G\textsubscript{\alpha i} simulation using the DynDom software [44, 45] (Fig 3). This analysis identified a rotational mode defined by a rotation of \(41^\circ\) of the G\textsubscript{\alpha i} protein around the rotational axis reported in Fig 3. This trend is not surprising as the initial conformation of AC5 in the binary complex originates from an AC:G\textsubscript{\alpha} structure in the absence of G\textsubscript{\alpha i}.

The root-mean-square fluctuation (RMSF) per residue was also calculated for the C1a and C2a domains in both the G\textsubscript{\alpha}-free AC5 and binary complex (S5 Fig). Concerning the C1a
domain, $G_{\alpha i1}$ reduces the flexibility of the C-terminal part of the $\alpha 3$ helix (residues 550-560) compared to $G_{\alpha}-$free AC5. This change in flexibility is not surprising since this region strongly interacts with $G_{\alpha i1}$, resulting in reduced mobility. In the C2a domain, the $\beta4^{-}\beta5$ strand (residues 1190-1200) is considerably less flexible in the holo AC5:G$_{\alpha i1}$ complex than in the apo binary and ternary AC5 complexes [27], indicating that ATP association can stabilize the mobility of the C1/C2 interface in holo AC5:G$_{\alpha i1}$ due to the positioning of the substrate.

**Ga$_s$ binding site**

A crucial site on C2 that could be affected by the conformational changes that occur within AC5, is the Ga$_s$ binding site. The effect of the presence of Ga$_{\alpha i1}$ with respect to potential Ga$_s$ association was investigated by comparing the conformations sampled by helix $\alpha 2'$ and $\alpha 3'$ (constituting the Ga$_s$ binding site) in Ga$-free$ AC5 and in the AC5:Ga$_{\alpha i1}$ complex (Fig 4). In the Ga$-free$ AC5 system, the $\alpha 2'-\alpha 3'$ distance (Fig 4) stabilizes around $\sim 1.7$ nm, thus showing an increase of about 2 Å with respect to the distance in the X-ray structure of the AC:Ga$_s$ complex (i.e. 1.48 nm, PDB ID 1CJK) [21]. However, when Ga$_{\alpha i1}$ interacts with AC5, this distance is significantly decreased from $\sim 1.4$ to $\sim 1.1$ nm.

This conformational change in AC5:Ga$_{\alpha i1}$ could potentially lower the probability of Ga$_s$ complexation due to a reduced accessibility of the Ga$_s$ binding site. A decreased $\alpha 2'-\alpha 3'$ distance was also observed in the apo AC5:Ga$_{\alpha i1}$ complex [27], suggesting that such conformational change is independent from ATP association.

Moreover, the suggested reduced ability of AC5:Ga$_{\alpha i1}$ to associate with Ga$_s$ indirectly proposes a favourable route towards the possible formation of a holo ternary Ga$_s$:AC5:Ga$_{\alpha i1}$ complex. These results suggest that the most plausible route to form a ternary complex would be to first form holo AC5:Ga$_s$ and subsequently bind Ga$_{\alpha i1}$, which was also suggested for the apo form [46].

**ATP binding site**

Although a reduced probability in Ga$_s$ association can negatively impact AC5’s catalytic activity as Ga$_s$ stimulates ATP conversion, sampling an ATP conformation that is able to convert
to cAMP is imperative for AC5’s catalytic function. The first part of the conversion of ATP to cAMP consists of the deprotonation of O₃⁻ (Fig 5), which is followed by a nucleophilic attack of O₃⁻ on the phosphorous atom of the α phosphate, Pα, producing cAMP. It is suggested that the first step of the reaction mechanism, the deprotonation of O₃⁻, would require O₃⁻ to coordinate to the neighbouring Mg²⁺ ion in the active site, which would lead to an O₃⁻-Mg²⁺ distance of ~2.7-2.0 Å [47–50]. Therefore, the distance between ATP’s O₃⁻ and Mg²⁺ plays a crucial role and was monitored along both holo trajectories to study the potential catalytic activity of the conformational ensemble of ATP (Fig 5).

In the Gaα-free AC5 system, the O₃⁻-Mg²⁺ distance was mainly found to oscillate around ~5.4 Å (5Å < O₃⁻-Mg²⁺ < 6Å is sampled in 75% of the trajectory), close to the X-ray distance of 5.25 Å (Fig 5C) [21]. To a lesser extend, a second conformation with an O₃⁻-Mg²⁺ distance larger than 6.0 Å (O₃⁻-Mg²⁺ > 6Å is sampled in 11% of the trajectory) was also sampled in Gaα-free AC5. This conformation is suggested to be inactive as the nucleophilic attack of O₃⁻ is unable to be performed at this distance (Fig 5D). Occasionally, the O₃⁻-Mg²⁺ distance decreased to a value lower than 3.5Å in the Gaα-free AC5 simulation (O₃⁻-Mg²⁺ < 3.5Å is sampled in 0.33% of the trajectory) (Fig 5B). Intriguingly, albeit poorly sampled along the simulation, this conformation could be close to the ATP structure undergoing the deprotonation step and can, therefore, be identified as the catalytically active or near-attack conformation, according to Hahn et al. [50].

AtP appears to adopt a different conformation in the AC5:Gaα₁ complex. The O₃⁻-Mg²⁺ distance increases up to ~8.0 Å in the first 300 ns, and equilibrates to a stable value of ~7.2 Å

**Fig 5. ATP sampled conformations.** The distance between ATP’s ribosyl oxygen O₃⁻ and the neighbouring Mg²⁺ is reported as function of time for both holo free AC5 and the AC5:Gaα₁ complex in image (A). The starting distance corresponding to the value found in the X-ray structure (PDB ID: 1CJK) is shown by a green dashed line. Snapshots of three representative conformations sampled by ATP and its neighbouring Mg²⁺ ions are shown in stick (for ATP) and ball (for Mg²⁺) representation and were extracted from the simulated trajectories of holo Ga-free AC5 at 500 ns (B) and the holo AC5:Gaα₁ binary complex at 0 ns (C) and at 1μs (D).
for the last 2.3 μs of the simulation (O3*-Mg2+ > 6Å is sampled in 96% of the trajectory), which makes the cyclization reaction unlikely to occur (Fig 5D). The absence of O3* − Mg2+ coordination seems to indicate an inhibited state of ATP in the binary complex as a near-attack conformation of ATP [21] cannot be sampled. Additionally, the main inactive conformation sampled by ATP in AC5:Ga_i1 adopts a structure incapable of sampling other states, whereas in the Ga-free AC5 system, an equilibrium between the different ATP conformations is already present on the microsecond timescale. In order to check the reproducibility of the observed conformational change of ATP in AC5:Ga_i1, a second MD simulation of the holo AC5:Ga_i1 complex was performed using different initial velocities. This simulation of ~0.7 μs confirms the presence of an inactive conformation of ATP in AC5:Ga_i1 since the same trend in conformational change was observed for the nucleotide as in the first AC5:Ga_i1 simulation (S6 Fig).

The inactive ATP conformation sampled in AC5:Ga_i1 could be the result of conformational changes at the C1/C2 interface, perturbing interactions between ATP and AC5’s active site.
When comparing AC5:Gαi and Ga-free AC5, a crucial difference seems to be a change in ATP’s interactions with the C2 domain around its adenosine group which takes place in the first 200 ns of the simulation. Whereas in the Ga-free AC5 system the interactions of the adenine moiety and the active site are stable (Fig 6), an irreversible loss of a hydrogen bond can be observed in the AC5:Gαi system between adenine and K1124, connected to a second hydrogen bond with D1198 (Fig 6, S7 and S8 Figs). The loss of the K1124-ATP and D1198-ATP hydrogen bonds appears to destabilize the position of the adenine ring in the active site, which reorients shortly after (Fig 6, S8 Fig). The conformational change of the adenine ring in turn seems to impact the interaction of ATP with Mg2+ as it appears to coincide with the increase in distance between O3’ and Mg2+, leading to the suggested inactive state of ATP (Figs 5 and 6). This mechanism was also observed in the second MD simulation of AC5:Gαi (S6–S8 Figs).

Both K1124 and D1198 are conserved across species (rat, dog, human, rabbit and mouse) and are also conserved between all membrane-bound isoforms of AC (AC1-9 in mouse). The conservation of these two residues in the active site could indicate that both residues indeed play important roles in maintaining the catalytic function of adenylyl cyclase. The performed simulations suggest that K1124 and D1198 are crucial for stabilizing the conformation of ATP in the active site and when these interactions are lost, an inactive ATP state is sampled.

Hence, these results suggest the conformational change of the ATP substrate into an inactive state is promoted by Gαi:AC5 complex formation. Although Gαi association appears to inhibit the catalytic function of AC5 destabilizing the active conformation of ATP, it cannot be excluded that Gαi could also affect AC5’s activity by impairing its substrate binding affinity. However, it was experimentally determined by Dessauer and coworkers [51] that Km does not significantly change upon Gα or Gαi binding. Therefore, the Gαi-induced conformational change of ATP in AC5’s active site seems to be the main effect of Gαi’s inhibition mechanism on AC5.

**Conclusions**

Here, the structural and dynamical properties of the AC5:Gαi complex have been characterized in order to understand the inhibition mechanism triggered by Gαi association. The conformation adopted by the ATP substrate in the binary complex significantly reduces the probability of an S_N2 reaction between O3’ and Pα, potentially preventing the formation of cAMP. These findings demonstrate that although ATP is able to interact with the C1/C2 interface in both AC5:Gαi and Ga-free AC5 systems, the catalytic activity of AC5 seems to be impaired by Gαi association, affecting the conformation of the substrate. Additionally, these results show that, as already observed in the apo form, also in holo AC5, Gαi association induces a conformational change in the Gα binding region on the AC5 C2 domain, thus resulting in a closure of this binding site. Based on this observation it is suggested that Gαi association prevents ternary complex formation via this route. This potential pathway, found in previous studies of the apo form, is also reported here for the holo form and confirms the existence of a potentially favourable route towards ternary complex formation from the AC5: Gαi complex.

**Supporting information**

S1 Fig. Secondary structures holo Ga-free AC5. Time evolution of the secondary structures of AC5 as obtained by DSSP analysis on the trajectory of holo Ga-free AC5. (TIF)
S2 Fig. Secondary structures holo AC5:Gαi1. Time evolution of the secondary structures of AC5 (top panel) and Gαi1 (bottom panel) as obtained by DSSP analysis on the trajectory of the holo AC5:Gαi1 binary complex.

(TIF)

S3 Fig. Hydrogen bonds analysis. Top panel: Number of hydrogen bonds (H-bond) present in the AC5 protein calculated on the holo Gα-free AC5 trajectory (black line), and in the AC5 protein (blue line) and Gαi1 subunit (green line) of the holo AC5:Gαi1 trajectory as function of time. Bottom panel: Time evolution of the number of hydrogen bonds formed between AC5 and Gαi1 along the simulated trajectory of the holo AC5:Gαi1 binary complex.

(TIF)

S4 Fig. RMSDs of Holo AC5 complexes. Root-mean-square deviation (RMSD), calculated on the protein backbone with respect to the starting structure of each protein subunit or the complete complex, is reported for holo Gα-free AC5 and the holo AC5:Gαi1 binary complex as function of time.

(TIF)

S5 Fig. Structural fluctuations. Root-mean-square fluctuations (RMSFs) of the C1 (top panel) and C2 (bottom panel) domains calculated on the protein backbone for both simulated systems, holo Gα-free and AC5:Gαi1, excluding the first microsecond of simulation and block-averaged over 100 ns segments. RMSFs of the apo AC5:Gαi1 system are also reported in cyan.

(TIF)

S6 Fig. ATP sampled conformations. The distance between ATP’s ribosyl oxygen O3\(^{-}\) and the neighbouring Mg\(^{2+}\) is reported as function of time in the first \(\mu\)s of simulation of holo free AC5 and holo AC5:Gαi1 complex. In the case of holo AC5:Gαi1 complex the same analysis was repeated on a second independent trajectory (blue line). The starting distance corresponding to the value found in the X-ray structure (PDB ID: 1CJK) is shown by a green dashed line.

(TIF)

S7 Fig. ATP’s adenine orientation in the active site of AC5:Gαi1 and Gα-free AC5. Distances between the adenine moiety of ATP and D1198 (A), D1198(N1)-ATP(N6), as well as K1124 (B), K1124(NZ)-ATP(N1), are shown for the two simulations performed for the AC5: Gαi1 system (blue and orange) and the Gα-free AC5 trajectory (black). The dashed green line indicates the value for each initial distance in the AC5:Gαi1 and Gα-free AC5 systems. (C) is a representation of an angle between ATP’s P\(_{\alpha}\) and two nitrogens in the adenine group, ATP PA-N9-N1, which are highlighted in Fig 6. This plot depicts the two simulations performed for the AC5:Gαi1 system (blue and orange) and the Gα-free AC5 trajectory (black). The dashed green line indicates the value of the initial angle in the AC5:Gαi1 and Gα-free AC5 systems.

(TIF)

S8 Fig. Conformational changes of ATP in the active site of AC5:Gαi1. Distances between the adenine moiety of ATP and D1198 (A), D1198(N1)-ATP(N6), as well as K1124 (B), K1124(NZ)-ATP(N1), are shown for the AC5:Gαi1 system for the first 200 ns of the trajectory. The dashed green line indicates the value for each initial distance in the AC5:Gαi1 system. The complete trajectory is represented in Fig 6. (C) Angle between ATP’s P\(_{\alpha}\) and two nitrogens in the adenine group, ATP(PA-N9-N1), is shown of the first 200 ns of the AC5:Gαi1 simulation reported in Fig 6. The dashed green line indicates the value of the initial angle in the AC5:Gαi1 and Gα-free AC5 systems. (D) Distance between ATP’s ribosyl oxygen O3\(^{-}\) and the neighbouring Mg\(^{2+}\) reported as function of time for the first 200 ns of simulation in the AC5:Gαi1 system. The complete trajectory is represented in Fig 6. The starting distance corresponding to the
value found in the X-ray structure (PDB ID: 1CJK) is shown by a green dashed line. (E,F,G,H) represent the respective values reported in (A,B,C,D) obtained in the second run of the AC5: Ga₁ system, which was simulated for ~0.7 μs in total (S7 Fig).

(TIF)

S1 File.

(ZIP)

Author Contributions

Conceptualization: Ursula Röthlisberger.

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Formal analysis: Daniele Narzi, Siri C. van Keulen.

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