Docking of ATP to Ca-ATPase: Considering Protein Domain Motions

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Most standard molecular docking algorithms take into account only ligand flexibility, while numerous studies demonstrate that receptor flexibility may be also important. While some efficient methods have been proposed to take into account local flexibility of protein side chains, the influence of large-scale domain motions on the docking results still represents a challenge for computational methods. In this work we compared the results of ATP docking to different models of Ca-ATPase: crystallographic apo- and holo-forms of the enzyme as well as "flexible" target models generated via molecular dynamics (MD) simulations in water. MD simulations were performed for two different apo-forms and one holo-form of Ca^{2+}-ATPase and reveal large-scale domain motions of type "closure", which is consistent with experimental structures. Docking to a set of MD-conformers yielded correct solutions with ATP bound in both domains regardless of the starting Ca^{2+}-ATPase structure. Also, special attention was paid to proper ranking of docking solutions and some particular features of different scoring functions and their applicability for the model of "flexible" receptor. Particularly, the results of docking ATP were ranked by a scoring criterion specially designed to estimate ATP-protein interactions. This criterion includes stacking and hydrophobic interactions characteristic of ATP-protein complexes. The performance of this ligand-specific scoring function was considerably better than that of a standard scoring function used in the docking algorithm.

INTRODUCTION

Prediction of binding of a ligand to protein target and the structure of their complex, referred to as molecular docking, is a powerful tool in understanding enzymatic mechanism and in drug design.[1,2] Many molecular docking algorithms have been developed that can more or less reliably dock a ligand into the protein active site. This approach implies that a high-resolution protein three-dimensional (3D) structure is available. For a large number of proteins, such structures in one or even more conformations have been obtained by X-ray or NMR studies. For other proteins, 3D models can be built based on known structure of their homologues. Based on the allowed flexibility of a protein and a ligand molecule, all docking methods can be divided into three groups: rigid ligand−rigid receptor, flexible ligand−rigid receptor, and flexible ligand−flexible receptor.

The early docking tools, the best known of which is DOCK,[3] were based on the key-and-lock mechanism of ligand−receptor recognition. This implies that both molecules are already adapted to each other before complex formation. In this approach the ligand and the protein were both considered as rigid bodies. While keeping the protein rigid, ligand flexibility was incorporated in the next generation of docking algorithms, the best known of which are GOLD,[4] FlexX,[5] Glide,[6] AutoDock,[7] and upgraded DOCK.[8] The rationale to keep the receptor rigid was that the ligand molecule as a rule has just a few rotatable bonds, while the protein has a vast number of such bonds. So, conformational sampling of the receptor would lead to an unreasonable increase of the computation time, and the compromise was that its flexibility was not considered. In any case, such an approach allowed considerable improvement of docking performance. Even if the active site was not able to bind the ligand in its initial conformation, the latter could now adapt to the protein structure.

On the other hand, structural studies of protein−protein[9] or ligand−protein[10] associations showed that these processes are often accompanied by conformational changes in protein structure. This effect is referred to as induced fit. Often these changes are restricted to protein side-chain conformations, although the main-chain motions of flexible loops are also observed. Moreover, for some proteins, changes upon ligand binding are not limited to local side chains and backbone. Instead, such changes can involve relative domain motions as in the case of, e.g., Ca^{2+}-ATPase.[11] These effects demonstrate the necessity of modeling target protein flexibility in the docking procedure.

In recent years a number of various approaches have been developed to incorporate receptor flexibility into a docking algorithm. Since this topic is widely covered by a number of excellent recent reviews,[12−16] we will just briefly consider the works most related to our study. One of the first proposed methods, which is referred to as "soft" docking, handles

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protein flexibility implicitly—by allowing the docked ligand to penetrate into the protein to some extent.[17] However, recent study showed that docking with limited, but explicit, protein flexibility results in "better" (in terms of root-mean-square deviation, rmsd) conformations of bound ligand and leads to higher enrichment in database screening compared to the "soft" docking algorithm.[18]

Other methods treat protein conformational changes explicitly and, as a consequence, are aimed at restricting the receptor conformational space to be sampled. For instance, the docking tool GOLD[4] allows limited protein flexibility by rotating only terminal hydrogen atoms of amino and hydroxyl groups to optimize hydrogen bonding. Such an approach essentially limits the conformational space of the receptor and permits treatment of protein and ligand flexibility in the course of docking simulations. At the same time, this seems to be useful only in cases of small conformational changes.

Full side- and main-chain flexibility is so far inaccessible for a docking procedure. The most common way is to use the standard docking scheme "flexible ligand−rigid receptor" with an ensemble of different protein conformations. Such an ensemble may be constructed of different crystallographic or NMR structures,[19−22] but it is highly dependent on the availability of structural data and on the range of conformational flexibility covered by these experimental methods. Other approaches use different simulation techniques to generate a discrete set of receptor conformations, thus reducing their dependence on initial experimental structures. Among these are molecular dynamics (MD) simulations,[11,23−30] which are also referred to as the "relaxed complex" method. Furthermore, a search for discrete low-energy conformations of side chains, which are stored in the rotamer libraries,[31−38] can be performed, or investigation of possible displacements of flexible loops based on graph and constraint theories.[39]

Some procedures have also been proposed that make use of such a discrete ensemble of protein structures during the docking procedure like in FlexE,[40] modified version of DOCK,[41] or SLIDE[42,43] software, combined protein grids method,[44,45] and "dynamic pharmacophore" model.[46] Docking by MD along with its modifications[47−54] is also worth mentioning here though this is not suitable for screening and can be used only in case of a small number of ligands.

Prediction of the influence of large-scale global conformational receptor changes on binding of a ligand still remains a challenging task. In the earliest attempt to take into account such receptor rearrangements, hinge-bending motions of protein domains were considered, while the domains themselves were held rigid.[55] Normal-mode analysis has also been used to sample large-scale loop motions of proteins.[56,57] Recently, a hybrid method combining MD and harmonic dynamics was applied to incorporate domain motions into the docking procedure.[52] As the availability of computational resources increases, it is now possible to perform long MD simulations of a protein to sample its conformational states for subsequent docking. However, as far as we know, this approach has so far very few examples of applications designed to investigation of the influence of global domain motions on docking results,[11] though it is widely applied to study global domain motions themselves.[58,59] Ca^{2+}-ATPase represents a suitable object for development and testing of such techniques. This enzyme belongs to the family of P-type ATPases and utilizes the energy of ATP hydrolysis to transport Ca^{2+} ions across the membrane.[60] Since in this study we are interested only in investigation of binding ligands and substrates, omitting the enzymatic function of target proteins, we will further refer to all of them as receptors. Aimed at detailed structural characterization of different stages of Ca^{2+}-ATPase reaction cycle, high-resolution structures of this enzyme, including complexes with ATP analogues, have been determined by Toyoshima et al.[61−64] and Sørensen et al.[65,66] The whole Ca^{2+}-ATPase structure is composed of four major domains. The transmembrane M-domain, that houses the Ca^{2+}-binding site, consists of 10 transmembrane alpha-helical segments (M1−M10). It is linked to three cytoplasmic domains (A, actuator or anchor; N, nucleotide-binding; and P, phosphorylation). The N-domain is inserted into the P-domain and these domains together are also referred to as the ATP-binding domain. ATP interacts with two active sites of this domain, one of which is located on the N-domain and the other on the P-domain.

Analysis of X-ray structures of the ATP analogue Ca^{2+}-ATPase complex shows that the ATP molecule binds to the N-domain via the adenine base and ribose, while phosphate tail is bound at the phosphorylation site of the P-domain. ATP can also bind solely to N-domain via adenine in the same orientation, as seen in the crystallographic model 1WPG.[64] This fact is also supported by the high-resolution structure of an isolated N-domain of Na^+,K^+-ATPase, a homologue of Ca^{2+}-ATPase, in complex with ATP as determined by NMR.[67]

All crystallographic conformations of the ATP-binding domain have similar structures of the N- and P-domains and differ mainly by their mutual orientation, which can be characterized by the angle between the N- and P-domains.[11] ATP simultaneously binds at two active sites on the N- and P-domains, which are well separated in the apo-form, due to relative domain rearrangement.[68] ATP analogues still can bind to the N-domain site, although with lower affinity.[67] Previous MD studies of apo-forms of Ca^{2+}-ATPase propose that the N- and P-domains undergo large-scale thermal motions independent of ATP binding and when ATP binds to the N-domain, these motions bring it in contact with the phosphorylation site of the P-domain.[11] The availability of high-resolution structure of the ATP analogue−Ca^{2+}-ATPase complex now allows comparison of the docking results with the experimental data.

The main goal of the present work was to investigate the influence of receptor flexibility on docking performance. Nowadays, a number of methods have been proposed for treatment of the local conformational changes related to rearrangement of protein side chains. On the contrary, the motions of the larger scale−flexible loops and mobility of hinge-bending domains represent a challenge for the docking methods, though some results have been reported. In this work we address the question whether one can simply use standard MD calculations of a protein structure for efficient modeling of these motions. This is demonstrated with the example of the ATP−Ca^{2+}-ATPase complex, where the ligand is bound simultaneously at two active sites residing on different domains. Meanwhile, in the apo-form the distance between these sites is roughly twice as large as the linear dimension of the ATP molecule. Therefore, using the X-ray apo-structures of Ca^{2+}-ATPase it is not possible to

Table 1. X-ray Structures of Ca^{2+}-ATPase Used in the ATP Docking Experiments

PDB entry	res (Å)	complexed with	interdomain angle Θ	refs
1WPE	2.70	adenosine diphosphate	111°	64
1T5T	2.90	adenosine diphosphate	111°	65
1T5S[a]	2.60	adenosine (β-γ methylene)-triphosphate	111°	65
1VFP	2.90	adenosine (β-γ methylene)-triphosphate	112°	63
1IWO[a]	3.10		125°	62
1XP5	3.00		155°	66
1WPG	2.30	adenosine diphosphate	157°	64
1EUL[a]	2.60		165°	61

[a] For these structures MD simulations were performed.

Table 2. Results of the ATP Docking into X-ray Structures of Ca^{2+}-ATPase

PDB entry	number of correct poses	best rank of correct pose by "goldscore"	best rank of correct pose by ATP-SCORE
1VFP	16	1	1
1T5T	6	1	1
1T5S[a]	3	7	1
1WPE	10	1	1
1WPG	1	20	1
1IWO[a]	1	33	11
1XP5	0	[b]	[b]
1EUL[a]	0	[b]	[b]

[a] For these structures MD simulations were performed. [b] No correct pose found.

predict the correct binding mode of ATP standard docking methods. To solve the problem, MD simulations were held in explicit solvent starting from three different X-ray structures of Ca^{2+}-ATPase. It was demonstrated that independently of the starting conformation it was possible to obtain correct docking solutions. Also, considering the issue of proper ranking of docking results we discuss some particular features of different scoring functions and their applicability to the "flexible" receptor model.

RESULTS AND DISCUSSION

ATP Docking into X-ray Structures of Ca^{2+}-ATPase: The Importance of Receptor Flexibility. A summary of the results of ATP docking to crystallographic structures of Ca^{2+}-ATPase is given in Table 2. The best results were obtained for the conformations corresponding to the "closed" state, which were taken from complexes with ATP analogues (1WPE, 1T5T, 1T5S, and 1VFP). In all of these cases ATP poses were found with rmsd from the experimental structure of less than 1.5 Å over adenine and less than 3 Å over the whole ATP molecule. For these enzyme structures more than three correct out of 60 available ligand conformations were found. All of them exhibit $\pi-\pi$ stacking between adenine and Phe487. Moreover, the hydrogen bond between the adenine amino group and Glu442 was observed in all cases except 1WPE—due to a slight displacement of the adenine in docking poses relative to the crystal structure.

Contrary to this, for other Ca^{2+}-ATPase structures, docking results were essentially different. For 1WPG, which was also taken from the complex with ADP, only one correct ligand conformation was found with rmsd values similar to those

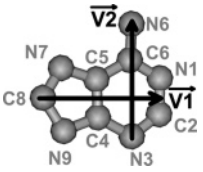

Figure 1. Adenine base. Two direction vectors associated with the structure are shown. Both of these vectors lie in the plane of the adenine and are used to check whether two ATP conformations are similar in the orientation of their adenine moieties.

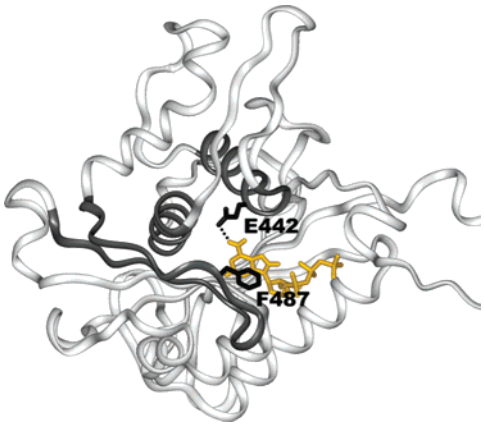

Figure 2. ATP-binding site at the N-domain of Ca^{2+}-ATPase (X-ray structure 1VFP). The residues Phe487, forming stacking interaction with adenine, and Glu442, forming a hydrogen bond to the adenine amino group, are shown. The loop of two β-strands (Met479-Pro500) and α-helices (Asp408-Cys420 and Glu439-Met452) forming the active site are shown in dark gray. Protein molecule is shown in a ribbon representation.

observed in the "closed" structures. Also, one correct ligand conformation was found for 1IWO, which represents an apo-form of the enzyme, though in this case the rmsd was higher: 2.5 Å for adenine and 5.9 Å for the whole ATP molecule. In both latter cases the hydrogen bond between the adenine amino group and Glu442 was not observed among docking solutions. This was mainly caused by different conformation of the backbone, which was not well-suited for adenine binding. Docking results for other "open" apo-forms (1XP5 and 1EUL) were even worse: no correct ATP conformations were obtained.

To understand the reasons for such differences in docking efficiency, one needs to compare in detail these structures of Ca^{2+}-ATPase. Thus, while Ca^{2+}-ATPase is able to bind ATP in the N-domain active site even in an "open" state, the local structure of this site differs from that in the "closed" X-ray conformations. Particularly this is related to the fact that in the crystallographic model 1WPG adenine does not form a hydrogen bond with Glu442 through its amino group, which is observed in other holo-forms.

This fact cannot be explained solely by inappropriate conformation of the Glu442 side chain. The issue is that the main chain adopts a different structure. As a result, slight conformational changes may have a dramatic effect on adenine-protein interactions. The N-domain ATP-binding site represents a hydrophobic cavity between two α-helices (Asp408-Cys420 and Glu439-Met452) on one side and a flexible loop joining two antiparallel β-strands (Met479—Pro500) on the other side (Figure 2). The hydrogen-bonding Glu442 is located on one of the α-helices, and Phe487 resides on the β-strand on the opposite side of the cavity. In Ca^{2+}-

ATPase structures corresponding to the "closed" holo-form this flexible loop is brought closer to the α-helices than in the "open" conformations (the distance between Cα atoms of Glu442 and Phe487 is larger by 1.5−3.0 Å), due to interactions between this loop and residues of the P-domain. Furthermore, the N-domain site adopts a "looser" conformation. Because of such differences in the backbone structure, adenine involved in stacking interaction with Phe487 is not able to form a hydrogen bond with Glu442. Based on these data, we decided not to take this hydrogen bond into account when classifying a given ATP pose as "correct" or "incorrect" (see Methods).

Up to this moment we were using rmsd as a criterion to select docking solutions, but the major task of docking is to select them by the values of a scoring function in the absence of information about the native structure of the complex. In this work we compare the ranking performance of the "goldscore" function[73] with an ATP-specific criterion ATP-SCORE.[75]

Docking yielded correct ATP poses for six of eight Ca^{2+}-ATPase structures (Table 2), but only in three cases they were top-ranked by "goldscore". Applying of the ATP-SCORE criterion brings the correct solution to the top in five of six cases failing only for structure 1IWO—the single apo-form among these six protein conformations. Besides the ability to rank the correct docking pose highest, the enrichment of the top-ranked poses by different scoring functions is interesting. We will illustrate this by the enrichment curves representing the fraction of all correct ligand poses ranked higher than a given cutoff value of the scoring function. As shown in Figure 3, the "goldscore" enrichment is rather good for the Ca^{2+}-ATPase structures. Nevertheless, ATP-SCORE performs clearly better. Particularly, this concerns the structure 1T5S, where the correct solution is top-ranked by ATP-SCORE, and occupies only seventh place when ranked by "goldscore". A very encouraging result is that in the case of 1WPG the only correct solution of 60 docking poses is ranked 20th by "goldscore" but ranks highest by ATP-SCORE. In case of apo-form 1IWO, none of these scoring criteria ranks the single correct ligand conformation at the top, but ATP-SCORE "raises" it from 32nd to 11th position compared to "goldscore".

Comparison with the X-ray data shows that the docking of ATP performs well mainly for protein structures that were taken from complexes with this ligand (the so-called re-docking procedure) but fails when the apo-form of the receptor is used. Similar conclusions have been made in other studies—this is referred to as the induced fit effect,[78] where the structure of the binding site is flexible and adjusts its conformation to the ligand upon complex formation. This is considered to be significant for nearly a half of studied protein targets[9] and requires taking into account flexibility of a target.

Overall, our approach to ATP docking to Ca^{2+}-ATPase with postdocking rescoring by ATP-SCORE shows quite good results for holo-forms of the enzyme. Even if ATP-SCORE fails to rank docking solutions, it considerably improves the enrichment characteristics. Still, to obtain comparable results for an apo-form, proper treatment of the receptor flexibility is needed. This conclusion concerns both the local motions of side chains and of flexible loops associated with the induced fit effect as well as large-scale

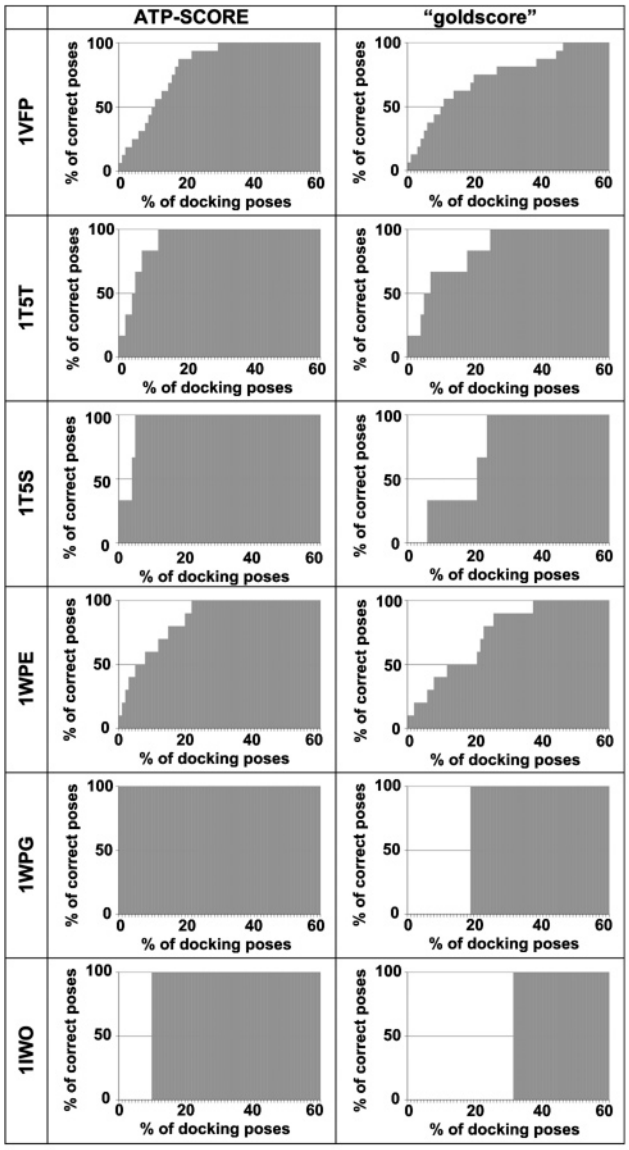

Figure 3. Enrichment plots for ranking the results of docking ATP into different X-ray Ca^{2+}-ATPase structures by ATP-SCORE and "goldscore".

domain motions, which represent an even greater challenge for computational methods. Thus, even if it is possible to predict the binding pose of ATP in the N-domain, as was done for 1WPG and 1IWO (in the last case more efficient ranking is still needed), docking to a rigid receptor does not allow correct prediction of ATP binding in the phosphorylation site of the P-domain as is observed in the experimental "closed" conformations of Ca^{2+}-ATPase.

Probing the Flexibility of ATP-Binding Domain via MD Simulations. To account for the flexibility of the enzyme, its conformational space was sampled by MD simulations. The resulting ensemble of protein conformations was then used in docking of ATP. This was done for three different X-ray structures of Ca^{2+}-ATPase. One of these structures is the holo-form 1T5S. The other two are the apo-forms with different extents of the opening of the interdomain interface: 1IWO (docking yielded one correct solution, although not the top-ranked one) and 1EUL (no correct ATP poses were found).

The RMSDs for the Cα atoms from the starting structure are ∼6 Å for 1IWO and 1EUL (results not shown). At the

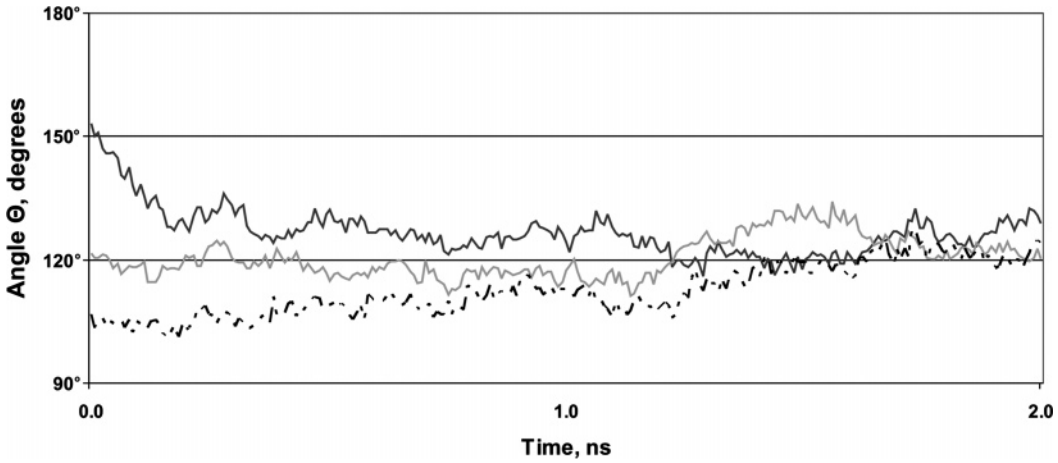

Figure 4. Dependence of the angle Θ on the simulation time for different starting X-ray structures of Ca^{2+}-ATPase. Each line color corresponds to different starting X-ray structure: dark-gray, 1EUL; black, 1T5S; light-gray, 1IWO.

same time, rmsd calculated separately over Cα atoms of the N- and P-domains are less than 3 Å. This indicates that MD simulations of all three Ca^{2+}-ATPase structures were exposed to large-scale domain motions, while conformation of the individual domains remained stable.

The essential dynamics analysis of the MD results permits delineation of low-frequency motions related to global conformational transitions. These represent movements of two dynamic domains with respect to each other. As determined by Dyndom software,[71] these protein parts correspond well to the N- and P-domains. The movements of the "closure" type provide the largest contribution to domain motions. This is consistent with differences in relative position of the domains in crystallographic structures. Nevertheless, the "twist" type motion has also significant contribution in the case of 1EUL. This is obviously due to the absence of the cytoplasmic actuator domain in our simulations. In crystallographic structures the latter is in close contact with the P-domain and in some conformations with the N-domain.

The relative orientation of the N- and P-domains plays a critical role in ATP docking since in X-ray structures of the complexes ATP is bound simultaneously to both domains. To describe orientation of the domains, the interdomain angle Θ was calculated along the MD trajectories (Figure 4). Angle Θ provides a good illustration of the domain orientation. While real hinge-bending besides "closure" motions also include "twist", the latter had little effect in MD of Ca^{2+}-ATPase. As was shown for the X-ray structures, in case when Θ is large, the distance between the two sites is large and ATP can bind only to the N-domain site. Meanwhile, when Θ decreases, the binding sites approach each other, and the ligand can bind at both domains simultaneously. Monitoring of the value of Θ along the MD trajectories reveals that starting from different relative orientations, all three Ca^{2+}-ATPase structures reach equilibrium in relative domain orientation after ∼1 ns of the dynamics run, the angle Θ oscillating approximately between 140° and 120°. Below, it will be shown that in case $\Theta \leq 120°$, it is possible to obtain such docking conformations of ATP that are bound to both the N- and P-domains. For 1EUL this happens during an interval of ∼0.4 ns, between 1.2 and 1.6 ns. For 1IWO this was observed during the first ∼1.2 ns and for 1T5S during almost the entire 2 ns. Thus, regardless of the starting Ca^{2+}-ATPase model, the angle Θ is observed to be lower than 120° within the first 2 ns for at least 0.4 ns in any MD trajectory.

Apart from the global domain motions, it is also interesting to investigate local changes in the backbone conformation in the vicinity of the N-domain active site. As noted before, the distance (*R*) between Cα atoms of Glu442 and Phe487 in the "open" conformations of Ca^{2+}-ATPase is larger than in the "closed" ones. This is one of the reasons that prevents the formation of hydrogen bond between the adenine amino group and Glu442. The value *R*, therefore, reflects an induced fit effect associated with backbone conformation. Monitoring the distance *R* along the MD trajectory reveals that it can either decrease or increase (Figure 5). In case of 1EUL it does not decrease significantly, thus explaining the fact that such a hydrogen bond was not observed in the docking solutions for this trajectory (see below). Meanwhile, in case of the other "open" structure, 1IWO, the value *R* decreases significantly at the beginning of the MD run thus allowing formation of hydrogen bond to Glu442. During the course of the MD run of the "closed" form 1T5S, this distance varies between the values observed in "open" and "closed" crystallographic models.

All these results indicate that during MD simulations closing of the interdomain interface of Ca^{2+}-ATPase was reproduced for both "open" X-ray structures. Nevertheless, the "closed" MD conformations exhibit particular differences compared to the experimental structures. Thus, moderate "twist" type domain motions were detected. This resulted in slightly different relative domain orientation in "closed" MD conformations. Besides that, in MD of 1EUL the main-chain conformation of the flexible loop of the N-domain binding site did not adopt the conformation more suitable for accommodating ATP. Taking all these factors into account, a conclusion was made that MD does not accurately reproduce all details of the "closed" form of Ca^{2+}-ATPase. Probably this is caused by the absence of the cytoplasmic actuator domain. Below we examine the influence of observed differences in Ca^{2+}-ATPase conformations on the docking results.

Docking into Ensemble of MD Conformations: Accounting for Receptor Flexibility. Docking of ATP was performed for all MD conformations of the receptor and compared with the experimental structures as described in

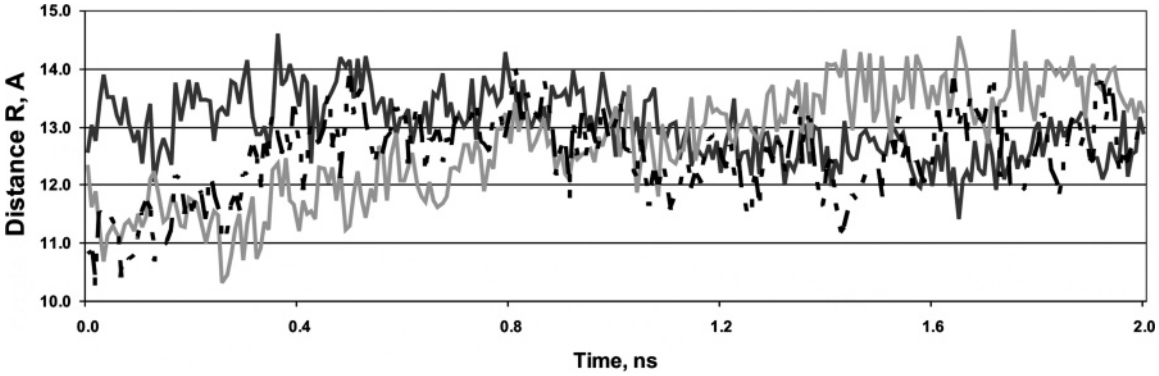

Figure 5. Distance between the Cα atoms of Phe487 and Glu442 in MD-conformations. Each line color corresponds to different starting X-ray structure: dark-gray, 1EUL; black, 1T5S; light-gray, 1IWO.

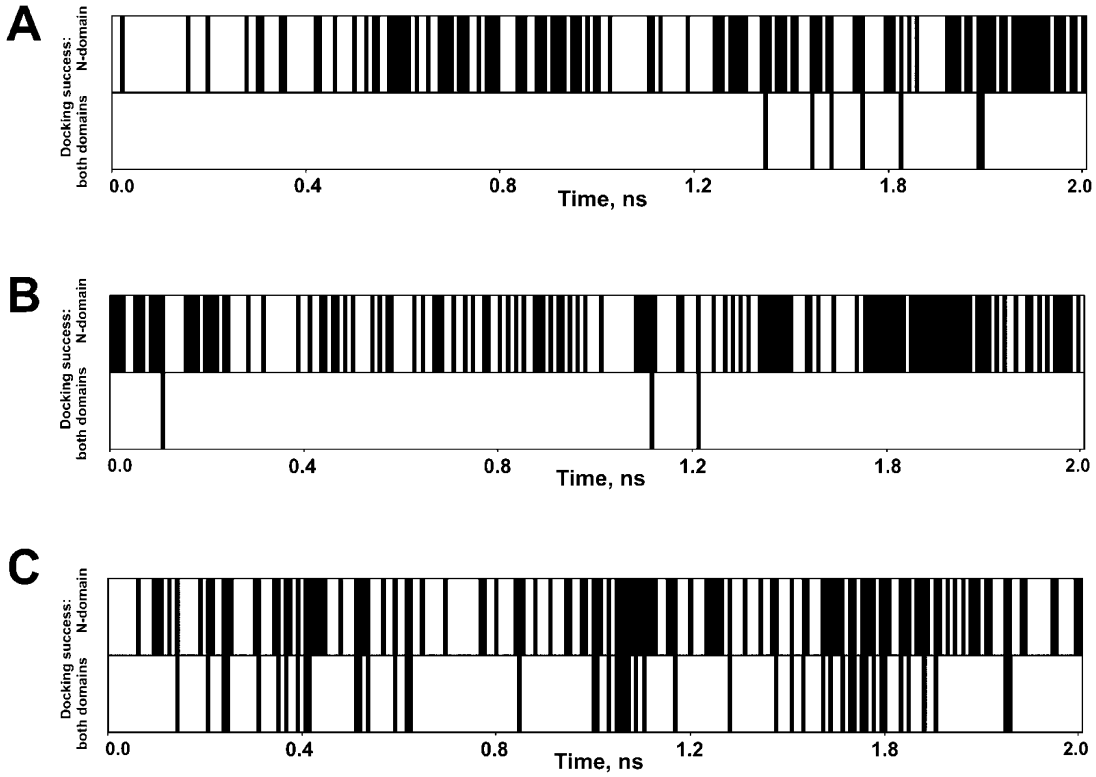

Figure 6. Success of docking procedure in obtaining the correct ATP conformation for MD-conformations of Ca^{2+}-ATPase: A, 1EUL; B, 1IWO; C, 1T5S.

the Methods section. In about 50% of the cases, correct adenine poses in the N-domain site were found. As shown in Figure 6, correct poses are distributed rather uniformly along the MD run. These results indicate that to obtain successful docking results in case of a single active site, there is no need to sample protein conformations with such small intervals (8 ps in our case) along the long-time MD run. It would be quite sufficient to perform sampling either with high frequency at rather short MD runs or with low frequency at long MD runs.

Actually, in the case of the apo-forms 1IWO and 1EUL the successful dockings are rarely found during the first ~0.5 ns of the run, which can be explained by the need for some kind of initial relaxation of the protein structure. Clustering of the MD-conformers based on the ability of an MD conformation of the protein to accommodate the ligand could reduce the number of target receptor structures and thus save the time of docking simulations. Unfortunately, we did not manage to find any rigorous criterion to estimate this property. Rmsd of the residues forming the active site did not provide such a criterion; similar problems have been also reported in other studies.[27] What we only found is that due to stacking between the adenine and Phe487, the conformation of its side chain can account for accommodating ATP to a moderate extent. The orientation of the Phe487 ring was described by the cosine of the angle between the normals of a given conformation and the "closed" crystallographic holoform. This value exhibited only correlation of ~0.2 with the success of docking.

At the same time, things are quite different when considering the influence of relative domain motions on the docking results. It is obvious that to obtain ATP poses bound to both domains via docking, the following conditions must be met simultaneously: the relative domain orientations should be appropriate and the local structure of both binding sites should fit the ligand. Thus, even though the periods of the "closed" domain orientation in MD trajectories intersect with many successful dockings to the N-domain, the number of

Table 3. Results of the ATP Docking into MD-Conformers of Ca^{2+}-ATPase

PDB entry of the starting structure	number of poses bound in P-domain site	number of poses bound in both sites (correct solutions)	best rank of correct pose by "goldscore"	best rank of correct pose by ATP-SCORE	rmsd of best solution by "goldscore" (over adenine), Å	rmsd of best solution by ATP-SCORE (over adenine), Å
1EUL	474	11	34	1	13.88	2.72
1IWO	384	3	150	1	11.92	1.45
1T5S	1771	87	38	1	5.52	2.74

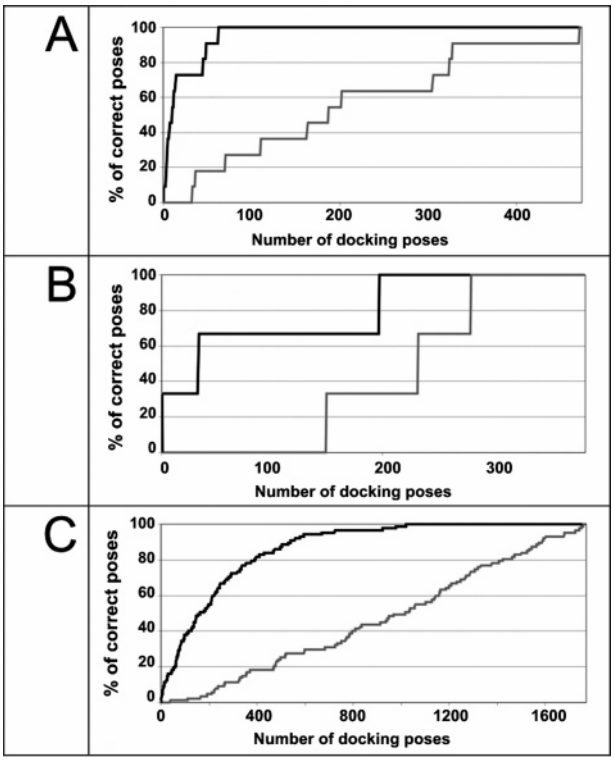

Figure 7. Enrichment plots for ranking the results of docking ATP into MD-conformations of different X-ray Ca^{2+}-ATPase structures by "goldscore" (gray) and ATP-SCORE (black): A, 1EUL; B, 1IWO; C, 1T5S.

complexes where ATP binds also to the P-domain is significantly lower. In the case of 1EUL, only for 11 protein MD-conformations were successful dockings to both domains obtained that correspond to the "closure" of the interdomain interface. Similarly, in the case of 1T5S, such dockings were often obtained as the interdomain interface was predominantly "closed" during the MD run. Surprisingly, in the case of 1IWO, only for three MD-conformations was such a ligand pose found, although the "lifetime" of the "closed" state is quite prolonged. Again, this is probably due to inappropriate local conformation of the binding sites. These results indicate that, in contrast to the case of a single active site, here a more efficient sampling of the receptor conformations is desirable or possibly further modifications to the docking algorithm would be needed to improve the success rate. For example, one may speculate that after sampling domain motions by MD, other algorithms could be applied to account for side-chain flexibility.

We investigated the influence of fast side chain and slow global domain motions on the results of docking. Conformational rearrangements of backbone regions (e.g., flexible loops) stand between these two extremes. For instance, in Ca^{2+}-ATPase this concerns the loop bearing the stacking Phe487. We discussed earlier that in the case of MD runs of 1IWO and 1T5S, this loop was found in a conformation similar to that observed in the experimental structures of "closed" holo-forms. Indeed, such ATP poses were obtained where adenine formed both the stacking contact to Phe487 and the hydrogen bond to Glu442. Still such results are rather rare for 1T5S, and in the case of 1IWO, only one such ATP pose is found. This is similar to the effect of domain motions. In MD of 1EUL, this loop does not adopt such conformations, thus resulting in a slightly larger distance *R* and an absence of docking solutions with the hydrogen bond to Glu442.

Yet it should be noted that in the cases of MD trajectories of 1EUL and 1T5S, the top-ranked ATP conformations exhibit a hydrogen bond between the adenine amino group and the Glu486 side chain. Glu486 is the residue next to Phe487. It resides in the active site side opposite to Glu442, which is seen to form a hydrogen bond to the adenine amino group in the experimental structures. This fact indicates that incorporating the receptor flexibility into the docking procedure not only may provide more correct solutions but also may give misleading ligand poses with high scores. Such an effect was also observed in other studies where receptor flexibility not only allowed improvement of docking with known ligands but also provided more possibilities for decoys to fit the receptor.[41,79] At the same time, analysis of those ATP conformations, which form a "wrong" hydrogen bond, showed that if the term of ATP-SCORE corresponding to hydrogen bonds between the adenine amino group and the protein was switched off, these ATP poses were ranked lower and leave at the top those poses without such a hydrogen bond. Therefore, one may conclude that the scoring criterion ATP-SCORE, which was designed on the basis of high-resolution crystallographic ATP-protein complexes, needs some adjustments to be applicable to less precisely determined receptor structures. This, of course, concerns the ensemble of protein MD conformations as well. Therefore, our results indicate that in this case incorporating hydrogen-bonding terms into the scoring criterion may be inadequate. Similar conclusions were drawn from a study dedicated to the analysis of the differences between optimization of the same scoring function separately for high- and low-resolution protein−ligand complexes.[80] That investigation also demonstrated that for less precise (low-resolution) receptor structures, the hydrogen bond term has much lower weight in the scoring function. Unlike directional hydrogen bonds, hydrophobic interactions, which are also rather delocalized on the molecular surface, seem to perform well in scoring docking poses for both high-resolution X-ray protein structures and for less precise receptor MD conformations.

Still, the ATP-SCORE criterion is not sufficient to select correct ligand poses since it is designed for scoring the placement of the adenine moiety of ATP only. To address the bonding in the P-domain site we considered only those

ATP poses with the distance between the γ-phosphate oxygen of ATP and the carboxylate group of the catalytic Asp351 less than 6 Å, thus yielding ~400 structures for 1EUL and 1IWO and ~1700 for 1T5S. These are rather diverse—rmsd of adenine from the reference crystallographic structure may reach 17.7 Å. Among them only 11 and 3 poses are correct in case of 1EUL and 1IWO, respectively, while there are 87 correct solutions for 1T5S (Table 3). This finding also indicates that the MD conformations of the protein produced from a holo-form are still more likely to accommodate the ligand. For all three trajectories correct solutions were selected with good enrichments by ranking the results with ATP-SCORE (Figure 7), which is comparable to the results obtained for X-ray structures of Ca^{2+}-ATPase. It is interesting however that the "goldscore" dropped significantly and resulted in almost random distribution of correct poses among misleading ones. This effect indicates that in case of the flexible receptor model more delocalized interactions such as hydrophobic contact implemented in ATP-SCORE provide better ranking of docking solutions. In this view apo-forms and MD-conformers exert similar influence on the performance of scoring functions, since in both cases the conformation of the binding site may be not optimal for accommodating the ligand.

CONCLUSIONS

We have studied the effect of different scales of protein flexibility, including global domain motions, local conformational changes of flexible loops, and side-chains flexibility on the results of ATP docking to Ca^{2+}-ATPase. Our results support the idea that local conformation of the active site may have a dramatic effect on the results of docking due to the induced fit effect. The ability of MD simulations to account for such effects is demonstrated by docking of ATP to an ensemble of generated MD conformations of the receptor.

Also, our study addresses the domain motions of Ca^{2+}-ATPase associated with the formation of complex with ATP. ATP interacts with two active sites of Ca^{2+}-ATPase located in different protein domains. While in the apo-form, the distance between these two sites is twice as large as the dimensions of the substrate, the holo-form is characterized by the closing of the interdomain interface which allows ATP to bind to both sites simultaneously. We performed MD simulations for three models of Ca^{2+}-ATPase with different domain orientations and demonstrated that regardless of the starting structure, it is possible to find a domain orientation similar to the "closed" state of the holo-form in each trajectory. Although MD did not provide all the details of the "closed" structure, this approach made it possible to reproduce the structure of the complex with rather good agreement with the experimental data.

Additionally, the problem of proper scoring of the docking solutions is addressed. We have applied our own scoring criterion, ATP-SCORE, developed especially for scoring ATP-protein complexes which showed better results than the general purpose scoring function implemented in the docking software. This improvement is probably because ATP-SCORE is based mainly on hydrophobic contacts between adenine and its binding site. Due to the delocalized nature of this interaction compared to, e.g., hydrogen bonds, it is not distorted by the induced fit effect or MD simulations. At the current stage, ATP-SCORE estimates binding of the adenine moiety of ATP only. Therefore, it has only limited application in this particular case as interactions of the ribose and phosphate tail are also of great significance.

COMPUTATIONAL METHODS

Ca^{2+}-ATPase Structures. MD simulations were performed for three Ca^{2+}-ATPase structures: 1IWO, 1T5S, and 1EUL (replaced by 1SU4). Docking of ATP were performed for MD-conformers and for eight high-resolution crystallographic structures of Ca^{2+}-ATPase (Table 1). Hereafter by the structure of Ca^{2+}-ATPase we mean its cytoplasmic ATP-binding domain, which in turn consists of its N- and P-domains. N- and P-domains together occupy an integral sequence range, the N-domain being an insertion into the P-domain sequence so that they are connected by a two-stranded hinge. All of these structures are different in the relative orientation of the N- and P-domains, which corresponds to the "opening" of the interdomain interface and can be quite well characterized by an angle Θ. The angle Θ is computed as the angle between two vectors connecting the center of mass of the hinge region (Cα atoms of Asn359 and Pro602) with the centers of mass of the most stable core of the secondary structure elements of N- (Cα atoms of Phe407-Thr554) and P- (Cα atoms of Glu606-Ile639 and Leu663-Ile743) domains.

In Table 1 all structures of Ca^{2+}-ATPase are sorted according to their value of Θ. The conformations with $\Theta \leq 120°$ (1WPE, 1T5T, 1T5S, and 1VFP) are referred to as "closed" and otherwise "open". The reason for such classification is the possibility of a protein conformation to bind ATP in both active sites simultaneously as will be discussed in more detail in the Results and Discussion section. All "closed" structures represent the holo-form (complexed with ATP analogues), while almost all "open" structures are apo-forms. Among "open" structures there is one with ADP bound only in the N-domain site. This is because the distance between the two active sites is too large.

MD Simulations. MD trajectories of the models 1EUL and 1IWO were taken from our previous work.[11] MD simulations of Ca^{2+}-ATPase ATP-binding domain extracted from 1T5S were carried out using the GROMACS 3.14 program[69] and the GROMOS96 force field[70] with the same protocol as was used for 1EUL and 1IWO. The protein with uncharged N- and C-termini was placed in a rectangular box of a simple point charge (SPC) model of water with edges of 10 Å from the solute. The 3D periodic boundary conditions were imposed. To keep the system elecrostatically neutral, 2 counterions (Na^+) were added. The energy of the system was minimized using 300 steepest descent iterations with fixed protein atoms followed by 300 conjugate-gradients steps with fixed backbone and final relaxation of 300 conjugate gradients steps without constraints. Then the system was subjected to a 10 ps MD run in an NPT (constant pressure and temperature) ensemble with fixed protein atoms, and then it was heated from 5 to 300 K during 60 ps in an NVT (constant volume and temperature) ensemble. Finally, an unrestrained 2-ns NPT MD production run was performed at 300 K so as to allow investigation of domain rearrangements. MD-conformations for subsequent docking were

extracted with the interval of 8 ps from the first 2 ns yielding in total 251 protein structures for each trajectory. Nonbonded interactions were truncated with the double cutoff of 10/18 Å. Processing of MD trajectories (including essential dynamics analysis) was performed using the GROMACS software. The Dyndom program[71] was used to delineate dynamic domains and to define the parameters of their movements.

Docking of ATP. Docking of ATP was done using the GOLD 2.0 program[72] with the scoring function "goldscore".[73] This program allows full torsional flexibility of a ligand, while flexibility of a receptor is restricted to terminal hydrogens in hydroxyl and amino groups. The parameters of the "genetic algorithm" were taken as standard default. The docking sphere radius was 21 Å, and the origin of this sphere was placed at the atom CD1 of Phe487. For each receptor structure, 60 independent docking runs were performed. Sybyl software (module Builder)[74] was used to add hydrogens to protein structures and convert them to MOL2-format (all ligand and water molecules were removed from the receptor structures).

Since some of the X-ray structures were complexed with ATP analogues, we estimated the success of a docking prediction be the rmsd over the adenine heavy atoms. The adenine moiety was chosen because it is responsible for specific binding of ATP in the N-domain. Due to the rigidity of the adenine structure, correct prediction of the position of the base also implies rather correct placement of the ribose. Nevertheless, adenine is a rather small structure (its characteristic dimensions, i.e., distance between atoms N6 and N9, are ∼5 Å), and if the rmsd is comparable to this value, then one cannot say whether this is due to errors in positioning of the base or it is related to errors in its orientation. To overcome this ambiguity we introduced two vectors associated with adenine which characterize its accommodation in the binding site (Figure 1). Adenine was considered placed correctly if its rmsd was less than 1.5 Å or if it was less than 3 Å and at the same time the angles between corresponding vectors in the two ligand poses were each less than 60°.

In structures 1IWO, 1XP5, 1EUL, and all MD-conformations, the reference position of ATP in the binding site is unknown. In these cases the N-domain of 1VFP was fitted onto these structures and the ATP associated with 1VFP served as the reference. The superpositioning was performed over Cα atoms of the secondary structure elements of the N-domain to exclude the influence of flexible loops motions. This, of course, should be considered as just a rough correctness check as the secondary structure packing of the N-domain may differ slightly in the Ca^{2+}-ATPase structures considered (especially in MD-conformers).

We should also note that when classifying any given ATP pose as "correct" or "incorrect", the presence of the experimentally observed hydrogen bond between the adenine amino group and the side chain of Glu442 was not taken into consideration. The reason for this is discussed in the Results and Discussion section.

Reranking the Docking Solutions. Docking poses generated with the GOLD software[72] using the "goldscore" scoring function[73] were then reranked by the ATP-SCORE criterion proposed earlier.[75] It takes into account typical adenine-protein interactions in ATP−protein complexes, including stacking, hydrogen-bonding, and hydrophobic complementarity. The strengths of the stacking and hydrogen bonds were computed based on their geometric parameters such as distances and angles. The hydrophobic properties of both ATP and protein were separately calculated at the Connolly surface[76] of ATP by the molecular hydrophobicity potential (MHP) method.[77] The strength of the hydrophobic contact is calculated as the ratio of the surface of matching hydrophobic regions of adenine and receptor to the total hydrophobic surface of the adenine and the binding site.

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