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Locating binding poses in protein–ligand systems using reconnaissance metadynamics

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A new, molecular-dynamics based protocol is proposed for finding and scoring protein–ligand binding poses. This protocol uses the recently developed reconnaissance metadynamics method, which employs a self-learning algorithm to construct a bias that pushes the system away from the kinetic traps in which it would otherwise remain. The exploration of phase space with this algorithm is shown to be roughly 6–8 times faster than unbiased MD and is only limited by the time taken to diffuse about the surface of the protein. We apply this method to the well-studied trypsin–benzamidine system and show that we are able to re-find all the poses obtained from a reference EADock blind docking calculation. These poses can be scored based on the length of time the system remains trapped in the pose. Alternatively, one can perform dimensionality reduction on the output trajectory and obtain a map of phase space that can be used in more expensive free energy calculations.

Introduction

Understanding how proteins interact with other molecules (ligands) is crucial when examining enzymatic catalysis, protein signaling and a variety of other biological processes. It is also the basis for rational drug design and is thus an important technological problem. Ligand binding is primarily examined using X-ray crystallography experiments together with measurements of the binding free energies. Additionally, numerous computational methods have been applied to this problem so as to extract more detailed information. The fastest of these approaches are based on an extensive configurational search of the protein surface (docking), in which the various candidate poses found are scored in accordance with some approximate function which treats solvation, protein flexibility and entropic effects in some approximate manner. Free energy methods, based on either molecular dynamics (MD) or Monte Carlo simulations, can be used to calculate accurate binding free energies [1, 2, 3]. However, it is far more difficult to use these methods to search for candidate poses as the time scales involved in ligand binding are typically much longer than those that are accessible in MD. Thus one often finds that the ligand becomes trapped in a kinetic basin on the surface of the protein from which it does not escape during the remainder of the calculation.

We recently developed a new method, reconnaissance metadynamics, for increasing the rate at which high dimensional configurational spaces are explored in molecular dynamics simulations [4]. This enhanced sampling is obtained by using a Gaussian mixture model to identify clusters in the stored trajectory, the positions of which correspond to the kinetic basins in which the system would otherwise be trapped. A history-dependent bias function is then generated that uses the information obtained from the clustering to force the system away from the traps and into unexplored portions of phase space. In what follows we demonstrate how this algorithm can be used to examine the binding of benzamidine to trypsin, the first blind docking simulations based entirely on enhanced sampling simulations involving a bias potential.

Background

Extensive conformational search procedures combined with fast and simple scoring functions give a surprisingly good description of protein–ligand docking in a variety of systems. In fact, for a number of systems so called blind docking calculations can be performed in which the binding pose is found without using any experimental insight [5]. The two greatest, unsolved problems for this field are to find universal scoring functions and to develop protocols for incorporating protein flexibility [6]. These two problems are interlinked as an accurate scoring function must take the energetic cost of the conformational changes into account. Standard biomolecular force fields together with implicit solvent models provide the best approach for balancing these contributions. However, empirical and knowledge-based scoring functions often perform better for certain classes of problems and are thus frequently employed [7, 8, 9].

Simulations based on molecular mechanics force fields provide an alternative to simple docking calculations and both MD and Monte Carlo simulations have been used to locate sites with favorable interaction energy [10, 11]. Furthermore, recent studies have exploited power of modern computers to examine the process of ligand binding directly [12, 13, 14]. In these calculations the ligand is initially placed outside the protein and MD is used to find favorable binding sites. In the limit of long simulation time, the sites are visited according to the Boltzmann distribution and thus can be scored based on the amount of time the ligand spends at each site. This approach allows one to incorporate the protein flexibility, to treat the water explicitly and to use established techniques for improving the force fields. In addition, one can obtain dynamical information on the binding process as well as structural information. However, these calculations still use an enormous amount of computational time, and produce so much data that specialist tools are required for analysis. For example, the recent paper on the binding of benzamidine to trypsin by Buch et al [12] used 500 unbiased simulations of length 100 ns.

Using plain MD simulations for locating binding poses is expensive because kinetic traps prevent the ligand from diffusing freely over the whole protein surface during short simulations. This is a general problem in MD and can be resolved by using enhanced sampling methods. A number of such methods have been applied to ligand bind-
The reconnaissance metadynamics method (RMD) \cite{4} inserts the rich data that can be obtained from short MD simulations into a self-learning algorithm and thereby generates local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to protein flexibility. The CVs we chose are based on the distances between the $C_{\alpha}$, $N_1$ and $N_2$ atoms of the ligand (see Fig. 1) and 16 uniformly spaced points on the protein surface (see materials and methods). These distances are then transformed by using them in tandem with docking calculations \cite{34}. The reconnaissance metadynamics method (RMD) \cite{4} inserts the rich data that can be obtained from short MD simulations into a self-learning algorithm and thereby generates local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to protein flexibility. The CVs we chose are based on the distances between the $C_{\alpha}$, $N_1$ and $N_2$ atoms of the ligand (see Fig. 1) and 16 uniformly spaced points on the protein surface (see materials and methods). These distances are then transformed by using them in tandem with docking calculations \cite{34}. The reconnaissance metadynamics method (RMD) \cite{4} inserts the rich data that can be obtained from short MD simulations into a self-learning algorithm and thereby generates local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to. The reconnaissance metadynamics method (RMD) \cite{4} inserts the rich data that can be obtained from short MD simulations into a self-learning algorithm and thereby generates local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to protein flexibility. The CVs we chose are based on the distances between the $C_{\alpha}$, $N_1$ and $N_2$ atoms of the ligand (see Fig. 1) and 16 uniformly spaced points on the protein surface (see materials and methods). These distances are then transformed by using them in tandem with docking calculations \cite{34}. The reconnaissance metadynamics method (RMD) \cite{4} inserts the rich data that can be obtained from short MD simulations into a self-learning algorithm and thereby generates local collective coordinates that can push the system away from the kinetic traps it encounters. This saves one from selecting a small number of appropriate CVs at the outset and thus provides a way to perform simulations when the reaction mechanism is uncertain. Thus far we have applied this method to model systems for polypeptide folding \cite{4} and to
space of collective variables. This is in contrast to methods like metadynamics, where the use of small number of collective coordinates forces these transitions to occur.

In an RMD simulation it will take some time to generate sufficient bias to push the system out of a basin. The specific amount of time will depend on the basin’s depth and hence its kinetic stability. Low free-energy poses are usually narrow minima in the potential energy surface. These states will be both thermodynamically and kinetically stable. It may therefore be possible to find low free-energy poses by extracting the most populated clusters from an RMD trajectory. To explore this further, we analyzed the RMD trajectory frames using the method of Daura et al. [42] that is implemented in GROMACS’ g-cluster utility. This procedure ranks each trajectory frame based on the number of neighboring frames that are within 1 Å RMSD. The top ranked frame, together with all its neighbors, is then removed and the ranking process is repeated.

Fig. 3B shows that the clusters generated from the analysis of the RMD trajectories are much smaller than those generated from an analysis of the MD trajectories. This confirms that the MD simulations are spending a great deal of time (up to 30 ns) trapped at a small number of sites on the protein surface. In contrast RMD spends at most 0.4 ns in any given pose and is thus able to explore more of the protein surface. In addition, this analysis of the RMD simulations identifies the binding pose as important. In three of the five RMD simulations that found the binding site, the cluster corresponding to the binding site is the most populated, while in the remaining two the binding site is ranked second and third.

Fig. 3C provides further evidence that clustering of the RMD trajectory gives reasonable binding poses. In this figure we show the vacuum interaction energy between the protein and the ligand for the top 50 clusters (i.e. the most populated ones) from each simulation. This interaction energy neglects solvent and entropic effects but is still often correlated with the binding free energy [43]. Hence, the fact that the clusters found in RMD have consistently lower energies than those found in MD suggests that they correspond to more strongly bound conformations. Furthermore, if we examine all the frames in the trajectory we find that, in contrast with MD, the top clusters in RMD correspond to the structures with the lowest energies. There is no such shift in MD which suggests that in these simulations the ligand becomes trapped in many basins that do not have particularly low interaction energies. As such, the MD simulations are too short to express the relationship between the residence time in a given structure and its free energy.

The clustering procedure does not take into account the bias, and thus some of the well populated clusters might not correspond to minima on the unbiased free-energy surface. Hence, to probe the kinetic stabilities of the poses from one of the RMD simulations, we ran unbiased MD trajectories starting from the 136 most populated clusters. During these simulations we took the time spent within 2.5 Å RMSD of the initial configuration as a measure of the stability of the pose and found that 89 poses were stable for more than 100 ps, 25 were stable for more than 1 ns and 7 of them were stable for more than 5 ns. Out of these seven poses, one was the crystallographic pose and one was a similar pose in which the ligand was separated from the Asp-189 residue by a water molecule. In addition, this set of poses contained the S2 and S3 states that were identified as stable in the MD studies of Buch et al [12] (see table S2). Intriguingly, a stable pose (see Fig. 4) was found in a part of the protein surface that was deliberately not explored in the MD investigation in reference [12].

This configuration remains unchanged for 60 ns of unbiased MD and we predict that it is one of most stable interaction sites outside of the binding pocket. It is possible that, like the S2 site, it acts as a secondary binding site [44]. For the EADock calculations a similar analysis showed that only 8 of the poses generated were stable for more than 100 ps (see table S1) and that this set included the binding site, the S2 site and a similar pose to that shown in Fig. 4.

Dimensionality reduction. Clustering is one way of examining the data from an extensive sampling of a high-dimensional phase space, such as that obtained from unbiased MD or an enhanced sampling calculation. An alternative is to perform dimensionality reduction [45, 46]. This is an appealing way of examining ligand binding as the largest changes in the position of the ligand are those corresponding to motion across the two-dimensional protein surface so the data should lie on a low dimensionality manifold. Furthermore, a low dimensional representation of the protein surface is a useful tool for visualizing the kinetic information that can be extracted from MD based approaches.

Many dimensionality reduction algorithms work by endeavoring to reproduce the RMSD distances between the trajectory frames in a lower-dimensionality space [47]. Clearly the RMSD distances between the ligand in the various trajectory frames could be approximately reproduced in a three dimensional space as they will be dominated by differences in position of the center of mass of the ligand. To further lower the dimensionality of the projection requires one either to incorporate periodicity in the low-dimensionality projection to lower the importance of the long range connections. We recently developed the sketch-map algorithm [48] as a tool for analyzing trajectory data. This algorithm uses the second of the approaches to the problem as it endeavors to reproduce the immediate connectivity between states rather than the full set of distances between frames. This is done by transforming the distances in the high-dimensionality and low-dimensionality spaces using a sigmoid function. Hence, close-together points are projected close together, while far apart points are projected far apart but not necessarily at the same distance. The justification for this is that in many chemical problems we know that there are only a small number of escape routes from any of the basins in the energy landscape. Hence, in fitting projections we should focus on reproducing the information on the connectivity as we have evidence that this data is low-dimensional.

We used the RMD trajectories to produce the sketch-map projections because, unlike our MD simulations which didn’t visit the binding site, we have sufficient sampling in the RMD to build a reliable map. There is a great deal of redundancy in the description of the position of the ligand in terms of the distances between surface atoms. Hence, we instead used the coordinates of the ligand’s Cα, N1 and N2 atoms in a protein-centered frame of reference to record the high-dimensionality positions. Fig. 5 shows that the resulting two-dimensional map clearly separates the poses around the binding site from other low energy poses on the protein surface and that there there are specific pathways and channels that connect the various clusters. Moreover, Fig. 6 shows that, in the area around the binding site, we are able to separate the metastable sites described by Buch et al [12], in spite of the fact that some of them are rather close in space (center of mass separation of ~4 Å). This suggests that sketch-map is also able to describe the orientation of the ligand and that using multiple atoms to define the ligand’s position is worthwhile. The resolution can be further improved by constructing a map using only points that are close to the binding site (see Fig. S1).

Footline Author
We can use the projections shown in Figs. 5 and 6 to do a qualitative comparison between the results of our RMD simulation and the results of the extensive MD simulations by Buch et al. [12]. In agreement with the previous study there is a significant population in the S3 state and a pathway from this state to the binding pose that passes through the TS1, TS2 and TS3 transition states. There are also other pathways between the bulk solvent and the binding site that pass through TS2 and TS3. In particular, during six of the ten binding or unbinding events that we observed, the ligand passed through the TS2 state on its way to or from the binding site, which suggests that this is the main binding pathway.

Conclusions
Molecular dynamics with explicit solvent has enormous potential for predicting protein-ligand interactions, because it is based on a physically motivated and systematically improvable potential energy surface and because it incorporates conformational effects, solvent effects and entropic effects in a physically consistent manner. Its main drawback is that it is considerably more computationally expensive than using docking calculations based on a configurational search with approximate scoring functions. One reason for this expense is that there are many energetic basins on the surface of the protein which can kinetically trap the ligand and slow down diffusion. This problem can be resolved by using a simulation bias to force the system away from kinetic traps and to flatten the energy surface. However, the requirement to find a small set of CVs that describes all the potential traps makes it difficult to do this using many established methods. In contrast, in reconnaissance metadynamics we can use large numbers of collective variables and let the algorithm work out which linear combination of them best describes each trap. The procedure outlined in this paper can thus be used to tackle problems where conformational and solvent effects play a large role, which would be difficult to examine using standard docking. Furthermore, the method is considerably cheaper than unbiased MD.

Reconnaissance metadynamics simulations provide an extensive exploration of the low-energy portions of phase space. One can use this data to find the approximate locations for the various basins in the free energy surface or alternatively use dimensionality reduction techniques to create low-dimensionality maps of phase space. The fact that these maps are low-dimensionality allows one to re-explore the interesting parts of phase space using other, more quantitative, enhanced sampling algorithms. In future, we will use this idea to extract accurate free energies for the various binding poses found during the RMD simulations.

Materials and Methods

System setup and computational details. The simulations were performed using GROMACS 4.5 [49] and the PLUMED plugin [50]. We used the Amber ff99 force field [51] for the protein and ff14SB [52] for the ligand. Van der Waals parameters were taken from the corresponding amino acids (phenylalanine and arginine), and appropriate charges were calculated using a RESP fit [52] to a Hartree-Fock calculation with the 6-31G* basis set - a procedure identical to that described in reference [27]. Long-range electrostatics was treated using the particle mesh Ewald approach with a grid spacing of 1.2 Å. A cutoff of 10 Å was used for all van der Waals and the direct electrostatic interactions and the neighbor list were updated every 10 steps. All production simulations were performed in the NVT ensemble at 300 K and this temperature was maintained using the stochastic velocity rescaling thermostat [53]. To prevent the system from sampling fully solvated configurations we used a restraining wall that limited the exploration to configurations where the sum of all the switching functions between the Cα carbon and the points on the surface was greater than one. This wall only has any effect when the minimum distance between the protein and the ligand is greater than 12 Å and represents a relatively small perturbation of the underlying energy surface.

The trypsin–benzamidine complex (PDB id 1.8JA) [54] was used as the starting structure in this study. All histidines were protonated on the N, site other than the catalytic H57 which was doubly protonated. This protein was then placed in an orthorhombic simulation box that extended at least 7 Å from any protein atom. Prior to production a 10 ns NPT simulation, in which the protein atoms were initially restrained, was performed to equilibrate the system. Ten RMD production simulations were performed together with 10 MD simulations. These calculations were started from ten statistically inequivalent configurations which had ligand was was outside the protein. For each calculation we ran one RMD and one MD simulation. The initial state of the metadynamics was generated by sampling the binding site by 20 Å and running a short equilibration run. The remaining nine starting points were selected from the MD trajectory launched from the first point. In all these initial configurations the protein–ligand distance was greater than 10 Å. Furthermore, we visually inspected the starting configurations in order to ensure the widest possible spread of initial configurations.

RMD Setup. Relevant points on the surface of the protein were selected by constructing a graph which had all the Cα atoms at its vertices and connections between any pair of vertices closer than 14 Å. A heuristic algorithm was then used to find the minimum spanning tree. This procedure produces a uniformly distributed set of Cα atoms on the surface. For trypsin these were the Cα atoms of residues 19, 33, 37, 47, 60, 74, 92, 97, 109, 127, 149, 159, 164, 183, 193, 229 and 244. The switching function was set up so that its value for a test point moving along the protein surface (5 Å above it) changed smoothly from −1 when it was immediately above one of the surface points to +0.4 once it was above the neighboring surface point 14 Å away. For the reconnaissance metadynamics, data was collected every 0.5 ps, which was then clustered every 100 ps. The bias was constructed from the clusters that had a weight greater than 0.2 in these fits and by endeavoring to add hills of width 1.5 and height 1.4 kJ mol−1 every 2 ps. Hills were only added when the distance from one of the cluster centers (in the metric of that particular cluster) was less than 0.356 - a distance that, at variance with previous applications of RMD, was kept constant for the entirety of the simulation.

As discussed in the main text we can easily create a more finely grained representation of the space by increasing the number of CVs and thus increasing the cost of the calculation. It is not straightforward to quantify the scaling with the number of CVs that describes all the potential traps makes it difficult to do this using many established methods. In contrast, in reconnaissance metadynamics we can use large numbers of collective variables and let the algorithm work out which linear combination of them best describes each trap. The procedure outlined in this paper can thus be used to tackle problems where conformational and solvent effects play a large role, which would be difficult to examine using standard docking. Furthermore, the method is considerably cheaper than unbiased MD.

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Fig. 1. The benzamidine ligand with the atom labels used in the text.

Fig. 2. The extent to which the space is explored in the RMD and MD simulations. The results from ten RMD and ten MD simulations are shown and each column corresponds to one of the reference poses from the EADock calculation. The squares are colored according to the minimum RMSD between the trajectory frames and the reference pose. If the minimum RMSD from any pose is greater than 2.5 Å then we assume that it was not found during the simulation and color it white.
Fig. 3. Comparison of the exploration speed, cluster sizes and interaction energies for the MD and RMD trajectories. Panel A shows the fraction of the reference (EADock) poses found as a function of simulation time. A pose is found if the RMSD distance between it and the instantaneous position of the ligand drops to less than 2.5 Å. The average over all the RMD or MD runs (solid lines) is shown together with the standard deviation between runs. The averages obtained using cutoffs of 2 Å (dashed lines) and 3 Å (dotted lines) are also shown. This panel shows that RMD consistently explores space more quickly than MD. In B and C we show the results from the clustering calculations. Panel B shows the sizes of the top 100 clusters averaged over the ten MD and ten RMD simulations together with the standard deviations calculated for every 10th cluster. The clustering was done using a fixed RMSD cutoff of 1 Å. Hence, more diffuse clusters have fewer members. In reporting this data, we have multiplied the number of trajectory frames in each cluster by the time interval between the frames (10 ps) in order to get a residence time for each cluster. This figure clearly demonstrates that RMD is spending much less time in each pose, allowing a more efficient exploration of the configurational space. Panel C shows a histogram of the single point protein–ligand interaction energy for the central frame in the top 10 (shaded area) and top 50 (unshaded areas) clusters from each of our RMD and MD simulations. Also shown is the histogram (dashed line) calculated from all the frames in the trajectory. This panel demonstrates that MD fails to find the low interaction energy poses that are found during the RMD trajectories.
Fig. 4. The structure of the most stable pose found on the back of the protein. The distances reported in the figure are in Å and are averages over a 60 ns MD simulation.
Fig. 5. The two-dimensional, sketch-map representation of the configurations visited during the RMD simulations. The interval between the projected frames is 100 ps so there are ~20,000 points in this figure. The points are colored based on the minimum RMSD distance to the experimental binding site (exp) and the three other docking poses that are displayed displayed in the inset. The color scale only extends out to 10 Å so if a point is further away from all of the sites than this distance it is colored in grey. The red rectangle indicates the location of the binding pose - this area is shown in more detail in Fig. 6. The docked pose P13 is the S2 metastable state reported in reference [12], and P26 shares its pocket with the metastable state shown in Fig. 4.

Fig. 6. Detailed description of the binding site. A shows a magnification of the part of the sketch-map projection that corresponds to the binding site - the area highlighted by the red rectangle in Fig. 5. Points in this figure are colored if they are within 2.5 Å RMSD of a specified pose. The poses indicated are the binding site (exp), the two most stable EADock poses (other than the binding site) that we found in this work (P2 and P12) and the poses described in reference [12] (S3, TS1, TS2 and TS3; see table S2). This last set of poses are the points along the pathway that Buch et al found most frequently connected the fully solvated ligand to the experimental binding pose. B shows where each of these poses is on the protein surface, while C shows the same information in more detail. In B and C the ligand molecule is colored, using the scheme from A, to indicate which pose is being shown.