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Protein Interactions with their Environment

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During functioning, proteins interact with and influence their environment. The analysis of interactions of proteins with their environment is of crucial importance for an understanding of protein function. Here, we focus on two aspects of protein interactions:

The first topic of interest is the analysis of the protein-protein complexation behavior. In this context, we analyze the complexation and the impact of mutations on this process. As a model system we chose the bacterial ribonuclease Barnase with its natural inhibitor Barstar. Here, our specific interest is the formation of intermediate states along the reaction coordinate as well as the driving complexation force in the system. In agreement with experimental data, we found that the complexation process is mainly driven by electrostatic interactions. This dramatically reduces the conformational space of the approaching complexation partners, resulting in the formation of stable encounter complexes. From these intermediates, the final complex structure is promoted.

The second topic is the modeling of proteins interacting with surfaces in the framework of the ProSurf EU project. In this context our focus is the simulation of protein adsorption on gold surfaces in water. As a first step we evaluated a classical set of parameters derived by ab-initio calculations from our cooperation partners. In obtaining mean force profiles for all 20 amino acids by constrained simulations and comparing them to experimental results, we found reasonable agreement between experimental and computational results. Additionally these simulations allow us to retrieve information first about the amino acid orientation towards the surface during different stages of complexation and second the total free energy difference during adsorption for each amino acid. In our simulations a clear barrier, attributable to the final water layer, could be observed.

1 Introduction

Interactions of proteins with their environment are fundamental for understanding the mechanisms of biological and hybrid systems consisting of biological and inorganic compounds. For transient complexes, electrostatic steering has an important contribution¹ to the association of the proteins. This contribution depends on the distribution of charges across the complexation partners as well as on the properties of the surrounding solvent. Contrary to macroscopic systems, the solvent properties are not homogeneous and isotropic but therefore depend on the surrounding protein surfaces².

In biological circumstances, proteins interact not only with their counterpart but also with inorganic surfaces like bone. Compatibility with non-biological surfaces and classification of protein-surface interactions is of increasing importance for nanotechnology and drug design. Yet, a physical understanding for these interactions is currently lacking. The major target of the ProSurf^a EU project is the development of a toolkit allowing the characterization of protein-surface interactions. In this task we evaluated derived force field parameters in molecular dynamics (MD) simulations.

^a<http://www.s3.infm.it/prosurf/>

2 Potential of Mean Force

The Potential of Mean Force (PMF) along a chosen reaction coordinate allows the calculation of the free energy difference between two states. The method of choice in our systems is the evaluation of constraint forces^{3,4} in simulations with constrained distances along the reaction coordinate. In case of the Barnase-Barstar model system, we chose the distance between the Centers of Mass (COM) as our reaction coordinate. The COM distance of the amino acid from the topmost gold layer plane has been the choice in our gold-amino acid systems. 21 distances (27 in the gold systems) have been sampled with at least $4 \times 5ns$ simulations per distance. The obtained Mean Force profiles are integrated to their potential form.

3 Protein Complexation

3.1 Mutations and Setup

Our target of studies of protein complexation is the well known system consisting of a ribonuclease Barnase and its inhibitor Barstar⁵. To analyze the impact of mutations on the electrostatic steering, we mutated Lys27 and Arg59 on Barnase as well as Asp39 and Glu76 on Barstar to Alanine, as experimentally suggested by Ref. 6 & 7, in one complex of the crystal structure⁸. Simulations at various constrained COM distances were conducted while monitoring the constraint force on the complex constituents as well as the orientation of the water molecules during the simulation. The forces obtained from runs consisting of $5ns$ simulation time at different distances were integrated as described in Sec. 2.

3.2 Results

In our Potentials of Mean Force significant differences between wildtype and mutated complex can be observed. While $\Delta F \approx 60 \frac{kJ}{mol}$ is in reasonable agreement with experimental values⁶, the mutated complex shows negligible free energy differences from bound to unbound state compared to the wildtype suggesting a major contribution of electrostatic interaction to the complexation energy difference.

A second observation in our distance constrained simulations was the presence of stable dipole fields at separation distances (additional displacement along COM-COM vector of complexation partners) of 20\AA between complexation partners in analogy to findings in simulations with single peptides⁹. These fields could be observed in wildtype simulations, but not in those with mutants.

4 Protein-Surface Interactions

4.1 Parametrization of 111 Gold Surfaces

The gold surface used in our simulations has been parametrized with the following scheme developed by our ProSurf cooperation partners in Modena: The van-der-Waals interaction of gold atoms is carried by virtual sites in the plane of gold surface atoms. These virtual sites are placed in the geometrical center of each triangle formed by neighboring gold surface atoms. Electrostatic interaction is modeled with dipoles at the position of all gold atoms as described in Ref. 10.

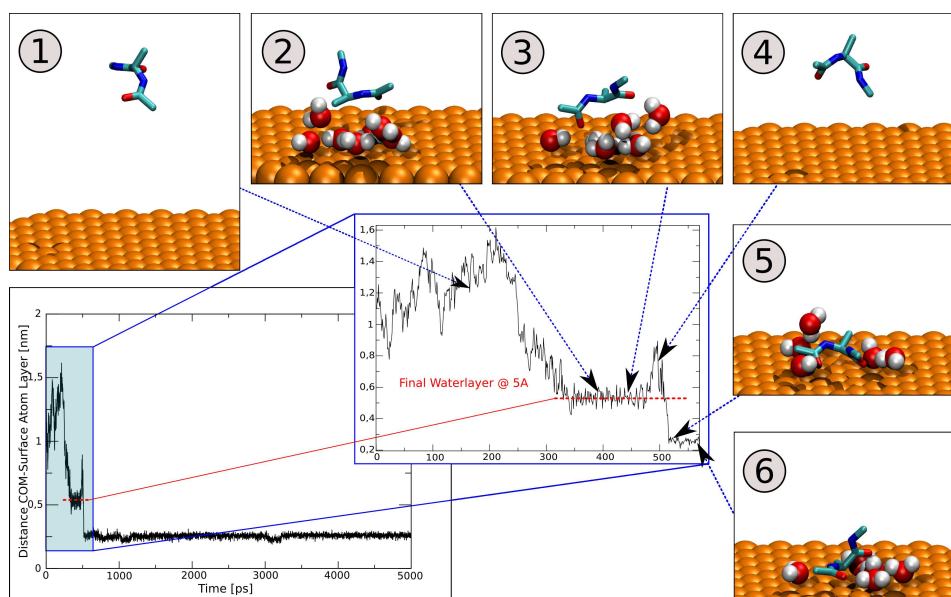


Figure 1. Adsorption process on parametrized gold surface of an amino acid (Alanine) with caps. The free simulation starts at a Center of Mass distance of $1nm$ from surface gold atom layer. A clear barrier at a distance of 5\AA is observable in the time-distance trace and attributable to the final water layer in the corresponding simulation snapshots. Only water within 5\AA of gold atoms and amino acid is shown in the snapshots.

4.2 Free Energy Calculation

In analogy to PMF calculation in Protein Complexes, we calculated Mean Force Profiles for amino acids with capped backbone. Our first results are in agreement with experimentally obtained values from Surface Plasmon Resonance (SPR) measurements and allow us further tuning of gold parametrization in 4.1.

4.3 Dewetting and Adsorption

To adsorb on the gold surface, the final separating water layer, as a barrier, needs to be overcome. Figure 1 shows a typical dewetting process of an amino acid. The barrier at a distance of 5\AA in the time vs. distance trace is clearly visible in frame (2) and (3) as the final water layer. The adsorption process in free simulations starting from $1nm$ distance above the gold surface is very fast for the uncharged amino acids ($\approx 500ps$) while the time until adsorption is significantly longer for charged and polar amino acids. This suggests different barriers during adsorption processes. When adsorbed on the gold surface, we could not observe desorption events in our simulation from any amino acid during $5ns$ simulation time.

5 Concluding Remarks

During complexation, water as the surrounding solvent can mediate a prealignment of the complexation partners, dramatically reducing their conformational space. This is even more surprising since the binding site of Barnase-Barstar is not more hydrophobic than the rest of the protein surface suggesting an increase of electrostatic steering instead of the hydrophobic effect as the major contribution to prealignment. Additionally, investigations on the adsorption process of amino acids on gold surfaces identify the final waterlayer as primary adsorption barrier.

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