Statement of Research and Teaching Interests

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INTRODUCTION

The continuous improvement of computer power and the developing of new fast algorithms [1] is rapidly increasing the possibility to use computer experiments to study the long term dynamics of large biomolecular systems [2]. Nowadays, atomistic simulations of biomolecular systems in solution, or in membrane environment, are limited to time scale of tens to few hundred of nanoseconds depending on the size of the system [3,4]. The use of less accurate physical model (coarse grained models) allows to extend these limits to larger molecular systems (e.g. ribosome [5]), and to complex processes like protein-protein recognition or aggregation processes (e.g. vesicle fusion [6]). However, these limits change rapidly and, in few years, we could reach a stage where the prediction of the protein tertiary structure will be afforded by direct folding simulations and complex protein recognition processes, involved in cell functions, will be studied at atomistic level. With this fascinating perspective in mind, my long term research interest and target is to use computational methods to afford meaningful simulations of molecular interaction processes at large scale level (e.g. protein folding, virus aggregation, chromatin organization, etc). In this respect, my research plan concern the continuation and further development of my current research interest towards this direction.

TOPICS

The various topics of my current and future research activities concern different aspects of computational chemistry and biology. These topics are not independent research units (as there is considerable overlap among them) and they converge to the aim of developing and applying accurate physical models to the description of large scale biological systems. My current research projects concern two main topics. The first one concern the comparison of the end-to-end encounter dynamics of small peptides with experimental time resolved fluorescent spectroscopy data provided by Prof. W.M. Nau at IUB. This study provides important information on the dynamics of peptides in solution and can be related to the mechanisms of proteins folding. The time scale of those processes is in the order of few tens of nanoseconds, that make possible, for the first time, a direct comparison with atomistic simulations [8]. The second aspect concern the study of dynamics of large protein and protein/DNA system. Among the different projects, molecular dynamics simulations of the nucleosome structure in water is the most challenging. The large system (more than 200000 atoms) require large computational power provided by the supercomputer at the Pacific Northwest National Laboratories at Richland, USA. This is the first Molecular Dynamics simulation study of this system and the aim is to analyze the dynamic behaviour of the DNA, histone proteins and the sorrounding water molecules.
My future research plan can be summarized as follows:

1. Study of stability and folding of biomolecules in solution.

Stability and folding of proteins constitute the main topics for the structural genomics part of my research activity. The study of the conformational dynamics of protein in solution constitutes the main step to the comprehension of the general mechanical properties of these systems.

Molecular Dynamics (MD) simulation methods (see [7,1]) offer the unique possibility to investigate these aspects at atomistic level. In this area, my main interests are focus on the following aspects:

- **Study of the stability and folding of peptides and proteins in solution.** The study of structural and dynamics properties of small peptides in solution can provide important insights into the mechanisms of protein stability, folding, and aggregation with relevant applications in medicine (as for example the formation of amyloid fibrils involved in neural-degenerative syndromes). In my research activity, I have afforded the study of the dynamics and kinetics properties of random and secondary structure forming peptides in solution as well as the effect of the solvent on these properties. The results of these studies have shown that short random peptides can reproduce the diffusive behavior that characterize the end-to-end contact of random peptides[8]. However, the absolute rate constants of this process resulted faster than the corresponding experimental values. Furthermore, it was founded that simulations with different force fields provide substantial differences in the calculated rate constants. This is not surprising since force fields for MD simulations are optimized to describe energy minima of conformation close to the crystal structure of folded proteins, but it also means that further optimization are necessary to refine them in order to get a more realistic description of the unfolding state and the folding kinetics. Nevertheless, long time simulations of peptides having the propensity to form secondary structures, have revealed the possibility to obtain secondary structure transitions (like the α-helix to β-hairpin transition of amyloid fibrils forming peptides [3]) on time scale of few hundred of nanoseconds. In the case of the folding of large proteins, the time scale of this process is still beyond the capability of conventional MD simulations. For this reason, new computational approaches to enhance the exploration of the protein conformational space are necessary [1]. I have used one these tools, the essential dynamics sampling, to study the folding of cytochrome c [13], obtaining results in qualitative agreement with experimental data. The use of this method allows to afford the folding process of medium size proteins on time scale of few hundreds of picoseconds. My future plan is to extend this approach to analyze the fold pathways of different class of proteins in order to find common features. Furthermore, the methods can be used in homology modeling or in structure refinements to improve the quality of protein models or of experimental structures.

  Solvent environment play also a very important role on the stability of secondary structure forming peptides in solution. I have performed studies on the effect of fluorinated solvents on the stability of α-helix and β-strand forming peptides that have shown, for the first time, as one of the mechanisms of co-solvent stabilization is related to a coating effect of co-solvent molecules that reduce the access of water molecules to the backbone hydrogen bonds of the peptide [9]. These interesting results encourage myself to extend the investigations on different peptides and proteins to better understand the details of this phenomena. My current researches are direct in this direction by studying wild type and mutants structures of cytochrome P450 BM3 in a mixture of DMSO/water in order to analyze combined effects of DMSO and mutations on the catalytic activity of this industrial relevant enzyme. These researches have important applications in bioengineering, where it is important for industrial reason to optimize the catalytic efficiency of the enzymes in solvent mixtures [10].

- **Study of the functional motion of biomolecules.** The next topic concerns the analyzes of the dynamic behavior of the folded protein machinery to understand its basic principles and its connection with the protein functions. I have performed simulation studies of dif-

ferent large enzymatic systems (the Citrate Synthase [11], the Glutamate Synthase [4]) and I'm currently involved in the study of the Nucleosome complex. In the case of the Citrate Synthase, the results have provided insights into the mechanism of domain closure induced by the ligand (oxalacetate) [11]. For Glutamate Synthase, the results have supported the hypothesis on the effect of the substrates on the activation of the enzyme towards the catalysis. In general, the interesting results from both these studies provide a further evidence (see also [2]) of the feasibility to get relevant information on the functional motions of large molecular systems (more 100000 atoms), with atomistic MD simulations. However, if the size of the system is too large or the time scale of the process to be analyzed is too long, then is still possible to use new methodologies (as essential dynamics sampling [12] or Steered Molecular Dynamics [2] to overcome these limits.

My future plan, concerning this topics is to extend the investigation to other proteins representative of different class of fold, in order to found common features in the dynamics of these processes and relate them to the structure of the proteins itself. The possibility to classify the protein dynamics allows to better understand its mechanism and to develop reduced protein models to afford the simulation of more complex systems like multi-domain proteins, the ribosome, chromatin and virus capsides.

2. Molecular recognitions in biomolecular systems.

The next step towards the simulation of the cell function is the study of the molecular basis of the biomolecular docking processes. There are several approaches to model protein-protein or protein-ligand interactions[14]. Most of these approach simplify the description of the interacting system by removing the internal flexibility for both the ligand and the protein, and by neglecting the presence of explicit solvent molecules in the calculation. These limitations restrict the use of these methods to cases in which the ligand is rigid and the conformational changes, induced by ligand on the protein, are small. The application of MD methods can overcame these limitations [15,16] by providing a more realistic description of the physical phenomena.
My future target in this area will be the use and the developing of methods based on MD techniques to study protein-protein or protein-ligand interactions. In particular, I'm interested to perform quantitative studies on the thermodynamics of ligand induced conformational change on the protein using novel approaches, like thermodynamic integration using Replica Exchange Methods [17], or potential mean forces calculations [2].

3. Course Grain Model approach to the description of large scale processes.

Finally, on a long run term, I'm planning to use the knowledge on the protein dynamics accumulated but the atomistic simulations in the point 1,2 and 3, to develop coarse grained protein and RNA/DNA models that could permit to afford simulations of very large scale process, like virus or ribosome formation, chromatin dynamics, motor proteins, on time scale accessible to the current computer technology.

FINAL REMARKS

I consider of fundamental importance for the realization of my research plan, the interactions with experimental groups at Indiana University This interaction will allow to have a direct evidences from the experimental data of the consistence and prediction of the computational models and provide a feedback to the interpretation of the experimental data. Furthermore, all those aspects of my research plan will require a great demanding of computational resources as well as the

improvement of the current force fields and methodologies. For this reason, a common basic aspect of this research plan will be the developing of new algorithms, the improving of simulation programs performance and the analysis of the simulation data by introducing graphical tools for the representation of the molecular information. In this perspective, my researches will also receive a great benefit from possible collaborations with theoretical and computational groups present at Indiana University. Finally, I am involved in a number of collaborations with colleagues from different EU countries and USA. I intend to reinforce these collaborations by promoting new projects for founding supports.

TEACHING INTERESTS

I enjoy teaching , and I look forward to this aspect of my academic career. My teaching philosophy is to transmit to students the enthusiasm and the curiosity that feed my interests in the study of Nature. I enjoy interactions with undergraduate and graduate students. I have experience in lecturing couses of computational chemistry and biology and a good experience in research mentoring. I have supervised the research activity of undergraduate and PhD students in the groups of Prof. A. Di Nola (at University of Rome "La Sapienza"). I have a wide range of teaching interests. I can teach any undergraduate class, any core graduate class and graduate courses in my areas of expertise. I look forward to giving back the knowledge I acquired through the years of my study, especially the topics related to computational chemistry and biology, bioinformatics, biophysics, structural biology, physical chemistry and programming.

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