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Biocomplexity Faculty Search Committee
c/o Professor Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
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December 12, 2003

Dear Professor de Ruyter van Steveninck and Search Committee Members:

With the letter I give my enthusiastic and unqualified recommendation for Dr. Marcos R. Betancourt who has applied for the Assistant Professorship position in your Institute Marcos worked with me for about three years after obtaining his Ph.D. from the Department of Physics at the University of California, San Diego. I am sure that Professor J. N. Onuchic, his thesis advisor, can elaborate on his accomplishments as a graduate student. Over the last few years Marcos has been nominally associated with Jeff Skolnick but working quite independently. I will confine my remarks to his accomplishments during tenure in our Institute and the impact his work has had in the intervening years.

Upon arriving at our Institute, Marcos promptly won the NSF minority postdoctoral fellowship that supported his research throughout his stay here. This was a feather in his cap. The fellowship allowed him to pursue quite independently many of his own ideas. My confidence in recommending Marcos highly comes because during his stay with me he accomplished a number of important tasks initiated largely on his own. In what follows I will describe the splendid work that Marcos has done in the area of protein folding.

Marcos has contributed to three separate areas in protein folding during his tenure as a postdoctoral fellow. One of them was the exploration of the limitations of the force fields required to obtain accurate structures. The common way of obtaining such potentials is through the analysis of structures in the Protein Data Bank (PDB) and with the use of Boltzmann distribution to extract energy scales for interaction between naturally occurring amino acid residues. Marcos showed that this could lead to errors. In order to minimize these errors he chose a completely different reference system that in addition to giving excellent correlation with experimental hydrophobicities also gives rellatively good energies for certain folded proteins. Although it was not designed for predicting structures Professor R. Elber and his coworkers (in a couple of papers published in *Proteins* during the last few years) have shown that this potential performs accurately when used in threading methods for structure prediction.

Marcos then used his energy function together with novel statistical mechanical methods to design sequences that will fold into specified structures. This has involved devising clever strategies for overcoming free-energy barriers in complex energy landscape. In what should be considered a very clever advance in Monte Carlo simulations of lattice and off-lattice models Marcos devised an ingenious method. It is known that multiparticle moves are necessary for enhancing sampling of conformations. Marcos showed that by considering large blocks of the molecules for which near-exact enumeration of the conformations are possible Monte Carlo efficiency could be greatly enhanced. This method is so powerful that we still use it in a number of applications in my research group.

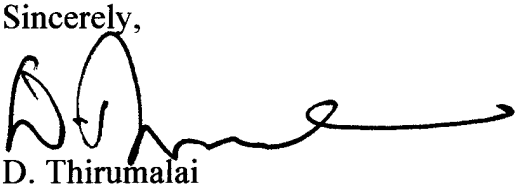
The second problem on which Marcos has spent considerable time is in the study of protein folding mediated by chaperones. It is well known that in many instances certain proteins require chaperonins to fold efficiently to the native state. Without this assistance the yield of the folded state is greatly reduced. Although there has been remarkable progress in getting X-ray structures of the chaperonins found in *E. coli* the mechanism of their action is not fully understood. In particular, there is considerable controversy as to whether the folding occurs while the substrate protein is encapsulated in the cavity provided by the chaperonin machinery. Because of the difficulties in interpreting these experiments Marcos devised simple computations to discover the workings of these biologically important molecular machines. Using controlled computations, he showed that in certain circumstances folding can occur inside the cavity provided there is sufficient change in the interaction between the walls of the chaperonin and the substrate protein after it is encapsulated in the cavity. The interaction has to change from being attractive to mildly repulsive. He also predicted that there is an optimum range of interaction between the substrate protein and the chaperonin for rate enhancement. These calculations also showed that chaperonin-mediated folding leads to global unfolding of the substrate proteins. Some of there predictions are already finding experimental support. These findings are not only important for chaperonin systems but they may also help understand recognition in other biological molecules.

There were two key predictions that Marcos made in his work on molecular chaperones. (1) As the cooperativity of the allosteric transition in GroEL (*E. Coli* chaperonin) increases, the rate at which substrate folds decreases. This unexpected prediction was confirmed experimentally by A. Horovitz in a paper that appeared in the *Proc. Natl. Acad. Sci.* subsequent to Marcos' paper in *J. Mol. Biol.* in 1999. (2) Marcos showed that the efficiency of the GroEL nanomachine increases if the turnover frequency increases and not if the time the substrate protein spends in the GroEL cavity increases. G. H. Lorimer and his student have beautifully confirmed this key prediction in a series of experiments.

The last problem he worked on here is the protein design or the inverse protein-folding problem. In this area one seeks sequences that will fold into preselected target structure. As such the solution of this problem will go a long way in being able to design drugs with targeted binding sites. Marcos has produced a novel strategy that enables unique design of structures that are stable and are also kinetically accessible. So far he has applied this strategy to semi-realistic lattice models with great success. I believe this approach holds great promise for applications to real proteins. This is an original piece of research that was independently conducted by Marcos. In this work he showed that no matter what the design temperature may be the sequences that fold most efficiently are those for which the collapse and folding transition temperatures are nearly the same. We are only now working on the evolutionary implications of this finding.

In summary, I feel that Marcos is extremely motivated to understand in great detail various phenomena in biomolecular folding using sound fundamental statistical mechanics. He is hard working and fiercely independent. This also gives him an obstinate streak, which should serve him well in the long run. He is easy to work with and listens well to good ideas. I have been very pleased to have him as a postdoctoral fellow in my group. I believe that he will greatly add to the already exciting intellectual atmosphere in your Institute. I feel that he would be a great asset as a research colleague. If I were you, I would grab him!

Sincerely,



D. Thirumalai
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