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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Department of Physics
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

RE: Dr. Florence Tama

Dear Committee Members,

I am very pleased to have this opportunity to support Dr. Florence Tama's application for a position with the Biocomplexity Faculty at Indiana University. Were Florence applying for a position here at Florida State University, I would be her biggest booster. I would love to have someone with her skills and interests here to help advance the use of computational methods to improve structures and elucidate mechanisms for large scale structural problems of the type studied by electron microscopy.

I first met Florence when I started my sabbatical leave at the Scripps Research Institute in August 2002 with Charles Brooks III. At the time I arrived, I was looking for exposure to computational methods in the hope that they would help to provide mechanistic insight to muscle contraction. I also wanted to see if incorporation of energetic considerations would help us to build better atomic models into low resolution cryoEM 3-D reconstructions. Florence was applying normal mode analysis to a model for the inhibited state of smooth muscle myosin that my laboratory had recently published and in her preliminary approach, she had taken the two heads of smooth muscle myosin that were in that model and showed that a single normal mode could conform one into the other. However, I was after somewhat bigger game and wanted to see if the method could provide some insight into the larger conformational change that occurs in the intact molecule when the two heads are held together by the S2 domain. Up to then, she had mostly worked with relatively compact structures, although with ribosomes and viruses, those structures are quite large. Myosin is to a first approximation, a couple of pear shaped heads connected to each other by an α-helical coiled coil "rope" that is about 10 times as long as the heads. This shape was quite a different problem from what she had previously attempted.

I built plausible active state models and she conformed them into the experimentally determined inhibited state structure. Although the experimental structure had a model for the S2 do-

main (the coiled-coil rope), we left the S2 out of the inhibited, target structure, but kept it in the starting, active state structure, to see where S2 would go if we just conformed the two myosin heads from the active to inhibited state. The surprise to me was that the S2 did not go where we had modeled it in the experimental structure, but to a far more plausible location that was better supported by experimental evidence. The modeling showed that the S2 location was coupled to the movements of the two heads.

That was not the only insight we gleaned from this effort. It had been known experimentally that formation of the inhibited state is sensitive to the length of the coiled-coil dimerization domain but it was not known why. You can form the needed 2-headed HMM molecule with just a leucine zipper, but it is not regulated. It was also known that smooth muscle myosin filaments are sensitive to the presence of ATP and in vitro, will spontaneously dissolve when you add ATP. However, it was not known why this occurs. We repeated the modeling using different lengths of coiled-coil, even out to the length found in whole myosin. By comparing the structural changes after conforming to the inhibited state, we found that the longer the coiled-coil, the smaller the changes but the changes propagate throughout the coiled-coil domain and may therefore affect the filament stability. This suggested that the α-helices of the myosin coiled-coil behave like torsion bars to this conformational change. We have the description of this work in press in the *Journal of Molecular Biology* that appeared on-line this week. In the smooth muscle myosin field, Florence's effort will have considerable impact but the work has broader implications for other 2-headed motors held together with a coiled-coil domain. Florence has done further work with me in modeling myosin structures in 3-D images of insect flight muscle and she has developed a program for using density maps as target functions for normal mode analysis. We have provided her with data with which to test it. I left her with a host of models to try so I am hoping that we continue long into the future. Obviously I really like what she does.

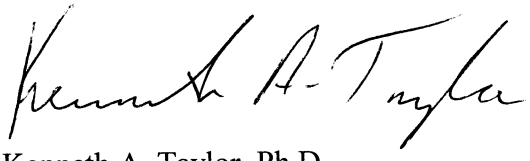
This experience convinced me of the importance of theoretical modeling methods for the understanding of complex biological structures and their associated functions. Tools like this are absolutely needed in electron microscopy where the resolution is often much less than atomic because of the inherent disorder of the system. This is an area that is neglected in the field. I think that her modeling of ribosome and virus motions are absolutely first rate. I hope that we can continue with the myosin modeling even after she leaves Scripps. Although I know she has other interests, if Florence continues to work in modeling large scale structures then I think she has a bright future.

Throughout the time I worked with Florence I found interactions with her were extremely easy and comfortable. She was patient with my relatively naive questions about what she does. She juggled multiple projects with much more skill than I would have. This suggests that she will be good at the multitasking that is necessary in an academic environment.

Among postdocs that I have known, Florence is clearly ready to move up to the next level. The only postdocs in the computational structural biology field I know well I met while on sabbatical in Brooks' lab. I had the good fortune to work with two of his best, Dr. Michael Feig and Florence. Florence's interests are different from Michael's, but I believe she is equally competent in her area of expertise. She is better prepared than almost any other postdoc with whom I am personally familiar and who have expressed interest in moving up to a faculty position. Florence is competent, hard working and ambitious and the combination will enable her

to succeed in an academic environment. However, because interactions with her are comfortable, I think that she will be a good colleague and carry her load. Like I said in the beginning of this letter, if she was interviewing here, I would be her biggest booster. If you need anything else from me, please let me know.

Sincerely yours,



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