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

Ref: Toby W. Allen, Ph.D.

Dear Prof. De Ruyter van Steveninck,

I write to express my enthusiastic support for Dr. Toby W. Allen, who is applying for an Assistant Professor position in your Institute. Toby received his graduate training in quantum field theory in the Department of Theoretical Physics at the Australian National University. For his post-doctoral training Toby shifted his research focus to the detailed computational modeling of membrane-spanning ion permeable channels, where he worked with Drs. Shin-Ho Chung and Serdar Kuyucak.

Toby's initial computational studies with Drs. Chung and Kuyucak were a systematic investigation of how the lining of an ion- or water-permeable pore would modulate the mobility of water and ions. This was followed by a massive computational effort where Dr. Allen and his colleagues developed the first complete description of ion movement through a KcsA potassium channel, work that later was extended to other cation-permeable ion channels. In this work Toby demonstrated his mastery of a wide variety of analytical and computational methods, which he combined into a hierarchical approach to understanding channel function – meaning that he combined molecular dynamics, Brownian dynamics, and analytical methods to deduce how ions might traverse valence-selective channels. As part of this work, Dr. Allen found that the deduced KcsA properties were dependent on the structure and dynamics of the phospholipids surrounding the channel.

Overall, this was a most productive period. Toby contributed importantly to the development of a hierarchical approach toward understanding the biophysics of ion permeation through membrane-spanning channels, and his results provided important new insights. This work put Toby "on the map." When we met at the Biophysical Society meeting in Boston in 2001 my appreciation of Toby only increased, and I invited him to join my laboratory and work with Dr. Benoît Roux and myself on ion permeation and channel-bilayer interactions. Toby was interested in becoming more conversant with the experimental situation, which he would achieve in my lab; he also wanted to approach the computational/theoretical studies with the methods that Benoît Roux was developing.

While at Weill Cornell, Toby has continued to be a very hard-working, productive investigator who has made many contributions. The initial focus was on channel-bilayer interactions, using the gramicidin A channel as the prototype, where we wanted to use molecular dynamics simulations to better understand how lipids would pack around the channel. Toby developed

robust, semi-automated methods for building the lipid bilayer around a channel, which overcame most of the deficiencies in earlier approaches; but as we were getting ready to go into production it became clear that we first had to resolve an important uncertainty related to channel structure. It turned out that two published high-resolution structures, one determined by solid-state NMR and one determined by solution NMR, differed with respect to both the backbone and some of the side chain orientations. The differing side chain orientation, in particular, could introduce serious uncertainties. Toby therefore decided to test whether molecular dynamics simulations could be used to identify the most likely structure, by calculating the NMR spectra from the molecular dynamics simulations and comparing with the experimental results. Toby could show that he indeed was able to use molecular dynamics to identify the most likely structure, which turned out to be the solution NMR structure. But the most important outcome of the study (which was published in *J. Am Chem. Soc.* earlier this year) was that neither of the NMR-derived PDB structures was all that successful in reproducing the measured spectra; it was only after relaxing the structures and allowing for molecular motion that it was possible to get good agreement with the experimental results.

Toby is good at multi-tasking, so while the above calculations were being completed he also began to address the question of ion permeation – as we now had a good channel model that moreover was imbedded in a lipid bilayer. Toby therefore could calculate both the one-dimensional and two-dimensional potentials of mean force, decompose the free energy profile, and finally estimate the channel's single-channel conductance. Calculating the potential of mean force is a challenge in light of the effective change in dimensionality that occurs when an ion enters or leaves a channel. Toby overcame the difficulties by considering the physics of the system, and calculating the energy of the system when the ion was in the pore and when it was in the bulk solution. By matching the results, he finally was able to produce a complete profile governing ion movement from one bulk solution to the other. Along the way he discovered a number of things, such as the need to correct for spurious electrostatic interactions that arise when simulating ion-permeable channels in a system with periodic boundary conditions, and the importance of correcting for the fact that molecular dynamics simulations are done in a medium of dielectric constant 1. Though this is well-known, it appears that Toby was the first to recognize that this could be a serious problem when simulating a bilayer-incorporated channel because the dielectric constant of the bilayer's hydrocarbon core in the calculations will be close to 1 (rather than 2, which is the experimental value), which has important implications for the calculation of the ion's self energy. Once all of these spurious effects had been properly accounted for, Toby could estimate the single-channel conductance to be ~0.8 pS, which is 30-fold less than the measured value; but nevertheless many orders closer to experimental results than any previous estimated based on molecular dynamics simulations. This is indeed a promising, and important, result.

But maybe the most important outcome of the calculations was the insights they provided into the importance of water for both ion translocation through the pore and the entry/exit process. When the potential of mean force was decomposed, it became apparent that the ion in the pore would reorient the water column, which had a major effect on the free energy barrier for ion permeation – confirming a prediction made by Kent Wilson some 20 years ago. Toby further could show how the incoming ions gradually lose their waters of hydration, and that the orientated water column could impose a barrier for ion entry that previously had been overlooked. Altogether this work (which in press in *Proc. Natl. Acad. Sci. USA*) provides important new insights into the physics of ion permeation.

Another important insight, which could not be fully presented in the above article, is the importance of molecular motion – a topic that already was broached in the *JACS* article. Toby decided to explore how conventional electrostatic calculations were sensitive to molecular motion. To do so he took a few nanosec of molecular dynamics trajectories and calculated the electrostatic potential profile along the channel axis every few picosec. The result was most

surprising, at least to me, as the electrostatic contribution to the free energy at any given point could vary by 20 kcal/mole. This work is presently being written up for publication.
As hopefully is clear from the above, I have enjoyed having Toby in the lab. He is a very hard worker, and he is not afraid of approaching difficult problems. His physical insights have contributed importantly to my own understanding of both ion permeation and channel-bilayer interactions. He is very helpful to everybody else in the laboratory, and he is well liked by all. But he is ready to move on and establish his own laboratory and research group. I have indicated to Toby that I would be delighted to continue to collaborate with him, as his insights and analysis will be invaluable for interpreting our experimental results; but this will be a collaboration of equals.

I cannot comment in detail on Toby's qualifications as a teacher/lecturer; but I have always found him to be clear and pedagogical in our journal clubs, and his research presentations are clear and aimed at a fairly general audience. I would expect that he would be a popular, if demanding, teacher of both undergraduate and graduate students. He certainly will put in the effort required to be a successful educator.

In summary, I have the highest regard for Dr. Toby W. Allen. He is ready to embark on his independent career, and he will continue to make major contributions to understanding the function of membrane proteins. I support with enthusiasm his application for an Assistant Professor position in your Institute.

Thank you.

Sincerely,

Olaf S. Andersen

Olaf Sparre Andersen