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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
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Swain Hall West 117
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To the search committee,

I am delighted to write this letter in support of Dr. Rahul Kulkarni's candidacy for a faculty position in your department. Rahul is a remarkably bright, energetic and creative scientist. His postdoctoral work in Ned Wingreen's group has played a fundamental role in our understanding of the underlying rules governing the regulation of gene expression and genetic networks in bacteria. I expect that as an assistant professor, he will continue to be one of the key innovators in combining the fields of biology, physics, and bioinformatics.

My laboratory investigates cell-cell communication in bacteria, a process called quorum sensing. Quorum sensing involves the production of extracellular signalling molecules called autoinducers. As a population of autoinducer-producing bacteria grows, the external concentration of autoinducer increases. When a threshold autoinducer concentration is reached, the bacteria detect the autoinducer, and initiate a signal transduction cascade that culminates in a change in behavior of the entire population. Quorum sensing has been shown to regulate a variety of physiological functions including: virulence, symbiosis, biofilm formation, antibiotic production and bioluminescence. Quorum sensing confuses the distinction between prokaryotes and eukaryotes because it allows bacteria to behave as multi-cellular organisms, and to reap benefits that would be unattainable to them as individuals.

Our genetic analysis of the quorum sensing circuits of *Vibrio harveyi* and *Vibrio cholerae* revealed that a protein called Hfq was the master regulator of the quorum sensing circuits. Hfq is known to interact with a variety of small non-coding RNAs (sRNA) to control translation of downstream target genes. We hypothesized that an sRNA must be involved in our circuit. Unfortunately, identifying these sRNAs by traditional *in vivo* genetic means has proven nearly impossible. Specifically, since the loci are only 50-100 nucleotides, one never obtains mutations in them in traditional screens. Because of this problem, we had planned an elaborate over expression experiment requiring much strain building, random screening of genomic libraries, etc. I expected

the identification and analysis of the sRNA locus to take us a year or so. Prior to initiating this work, I decided to ask Rahul to take a stab at identifying the locus encoding the sRNA using bioinformatics. He wrote an algorithm that allowed him to identify inter-genic sequences containing promoters that require the alternative RNA polymerase subunit σ^{54} (we knew σ^{54} was required from our earlier genetic studies), a terminator sequence, and that lacked a ribosome-binding site. Two days later Rahul presented me with a list of four putative loci that could contain an sRNA based on all of our criteria. Further evidence for his result was his finding that the four regions were highly conserved across all the available Vibrio genomes. The next day we performed a Northern analysis that showed that indeed one of the candidate sRNAs controls our quorum sensing circuit. Thus, with Rahul's help we did the work in three days! We have gone on to show that the other three candidate sRNAs are likely involved in quorum sensing, but to a lesser extent than the one I have described. I cannot adequately describe how important this result is to my group, or how wonderful it is to have the answer before we even began experiments to get it. In other collaborative work, Rahul has developed bioinformatic approaches to predict the DNA binding sites for two of our other quorum sensing regulators (LuxO and HapR). Again, his predictions have been borne out by our subsequent experiments. This later work has allowed us to define both the quorum sensing regulon and the wiring of the regulatory circuit in *V. harveyi* and *V. cholerae*. These are major goals of my group.

Rahul has been a wonderful collaborator. His work on the organization of gene networks has opened up entire new areas of investigation for my lab. His work gives us new ways to think about information processing, and importantly, his research has/will impact the fields of physics, biology, computational science, and engineering. Rahul's research is especially compelling because of his ability to work so closely with the experimentalists. He enthusiastically embraces the biological problems, independently and rigorously finds the theoretical solutions, and then understands and has good advice on the experimental issues. He appreciates that theory must be driven by the experiments and that, as a theorist, he must be closely tied to the experimentalists. He is very, very involved with my group. He does not simply sit in front of a computer. He's very often to be found in my lab talking with the students and postdocs about the experiments they are doing, giving suggestions based on his theoretical predictions, and using their results to further refine his models.

I hope you will consider Rahul's candidacy for a faculty position very favorably. As I've said, he is a gifted scientist, and furthermore he is also a delightful person. He is kind, generous, funny and extremely likable. He interacts well with everybody, and I know that in the future, he will foster collaborations and bring people from different disciplines together to solve problems in fundamentally new and exciting ways. Talking to Rahul invariably gives me new insights into my own work. I would support his candidacy for a faculty position in my own department without reservation. I hope you will do the same.

If you need more information, please feel free to contact me.

Sincerely,

Bonnie L. Bassler