



Howard Hughes Medical Institute
Research Laboratories

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Biocomplexity Faculty Search Committee
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Dear Committee Members:

Andrew Hausrath was a graduate student in my group. In a nutshell, he is a highly intelligent individual who worked exceedingly hard on a very difficult project, namely the determination of the structure of the F1 ATPase from *E. coli* at 4.4 Å resolution. In the process he developed an entirely new expression system for the F1 particle which, for the first time, provided large amounts of the protein for ongoing structural studies. He also has outstanding mathematical and computational skills which he had only limited opportunities to exploit in my group.

When Andy first joined my group he carried out crystallographic studies of thermolysin-inhibitor complexes as well as substitutions of the essential zinc ion at the active site. These led to two publications and, in the process, provided Andy with experience in the fundamentals of X-ray crystallography. He also developed a new crystallization system for the protein and, in the process, determined the structure of the protein in a new crystal form. This turned out to provide the first view of the protein in an "open" substrate-free conformation.

As his thesis project Andy decided to take on an exceedingly difficult challenge, namely to determine the crystal structure of the F1 ATPase from *E. coli*. As you will know, Walker's group at the MRC Laboratory in Cambridge initially reported the structure of the F1 ATPase from beef heart mitochondria. Although this work deservedly received high recognition it left many questions unanswered. Andy hoped that further insight could be obtained by studies of the *E. coli* enzyme which has been analyzed extensively by Professor Rod Capaldi of this Institute. The *E. coli* system is less complex than its mammalian counterpart. In addition, it has the great advantage that one can introduce mutations. In particular, the Capaldi laboratory has developed methods to engineer disulfide bridges that lock the enzyme in catalytically distinct conformations.

After an heroic effort, Andy was able to crystallize the wildtype *E. coli* F1 complex in a form that permitted determination of the structure at 4.4 Å resolution. This provided new information regarding the overall symmetry of the molecule and revealed the structure of parts of the γ -subunit not seen in the MRC structure. It also showed how the γ -subunit connects between the F1 ATPase and the proton-translocating F0 domain.

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In an important breakthrough for future work, Andy also developed an entirely new approach to obtaining large quantities of the F1 ATPase. The traditional purification protocol starts with the membrane-bound F1F0 particle. Andy has shown, in contrast, that large quantities of the F1 particle can be obtained by co-expressing the necessary subunits in the absence of F0. This breakthrough will be a major benefit to ongoing structural studies of the enzyme.

Andy's background is in mathematics and physics. At the same time, his experience as a graduate student has fully equipped him to handle all aspects of protein crystallography from cloning and expression through protein purification and crystallization to the structure determination and analysis of highly complex macromolecular assemblies. He was also an enthusiastic and helpful member of the laboratory. For example, on his own initiative he assumed the major responsibility for overseeing the installation of our MALDI-TOF mass spectrometer system, carrying out the initial tests, and instructing other lab members in the use of the instrument.

Recently, Andy has developed a completely new approach to represent a protein fold as a continuous curve which can be manipulated to describe and create protein models. This project will allow Andy to exploit his strength in mathematics, together with his expertise in structural biology. I will be very interested to follow his development of these novel ideas.

Andy has all the tools to be a highly effective structural and mathematical biologist. He is very bright and got on well with everyone. I expect him to be an excellent mentor and teacher and am pleased to recommend him strongly.

Yours sincerely,



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