

Outstanding Biomedical Research from CHESS Users

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Many macromolecular crystallographers come to CHESS to collect data, and many biologically important crystal structures result from these data. Here we turn the spotlight on two of these crystallographers, both of whom are working at the very forefront of biomedical research, and producing results of great scientific and medical interest.

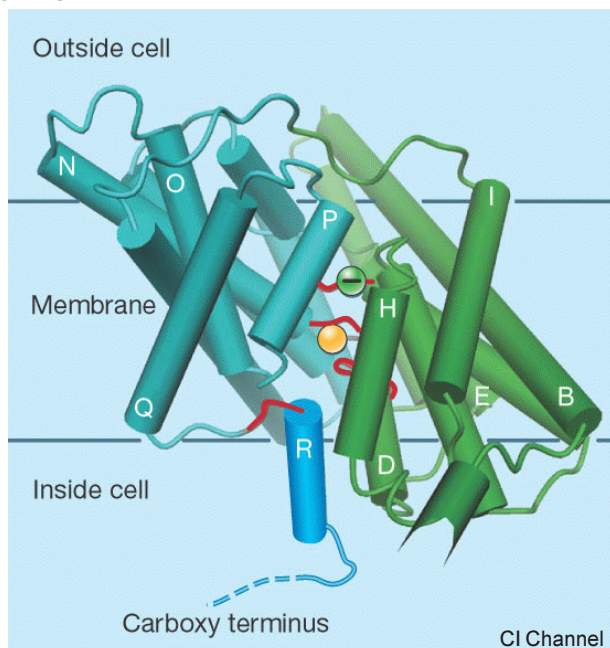
Rod MacKinnon

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Rod MacKinnon



When Rod MacKinnon first began coming to CHESS in 1997, he knew a good deal about the electrophysiology of ion channels, but little crystallography. That ignorance did not last long, as he threw himself into the extremely challenging project of determining the first structure of a transmembrane ion channel. After collecting massive amounts of data on numerous visits to CHESS and working through a number of crystallographic difficulties, MacKinnon and co-workers published the structure of the KcsA potassium channel from *Streptomyces lividans* in 1998 [1]. The importance of the structure to the study of ion transport was immediately recognized, and it was named a “Breakthrough of the Year” by Science magazine.



Since that first channel protein, MacKinnon has worked on many others; he is now one of the leading authorities on the subject of ion channel structure and function. As his crystallographic projects have expanded, so has his need for beam time; one synchrotron source is not enough and he is now a regular visitor to many of them. The MacKinnon group (usually including the indefatigable Rod himself) visits CHESS about 6 times a year, and is always welcome due to the expertise of its members, who by now know as much about operating the stations as the staff do. MacCHESS feedback forms for the group often report that data have been collected on a “novel ion channel protein”, as MacKinnon continues to expand his investigations.

The most recent major discovery from the lab is the structure of the chloride channel, a cover story in Nature earlier this year [2]. The structure (shown in the figure) reveals that this anion channel operates quite differently from the cation channels studied earlier. The K^+ channel has a single pore constructed from a tetramer of identical protein molecules, all oriented the same way relative to the membrane's surface, while the Cl^- channel has a “double-barreled” structure containing two pores, and the proteins making up the two pores are inserted in the membrane “upside-down” relative to each other.

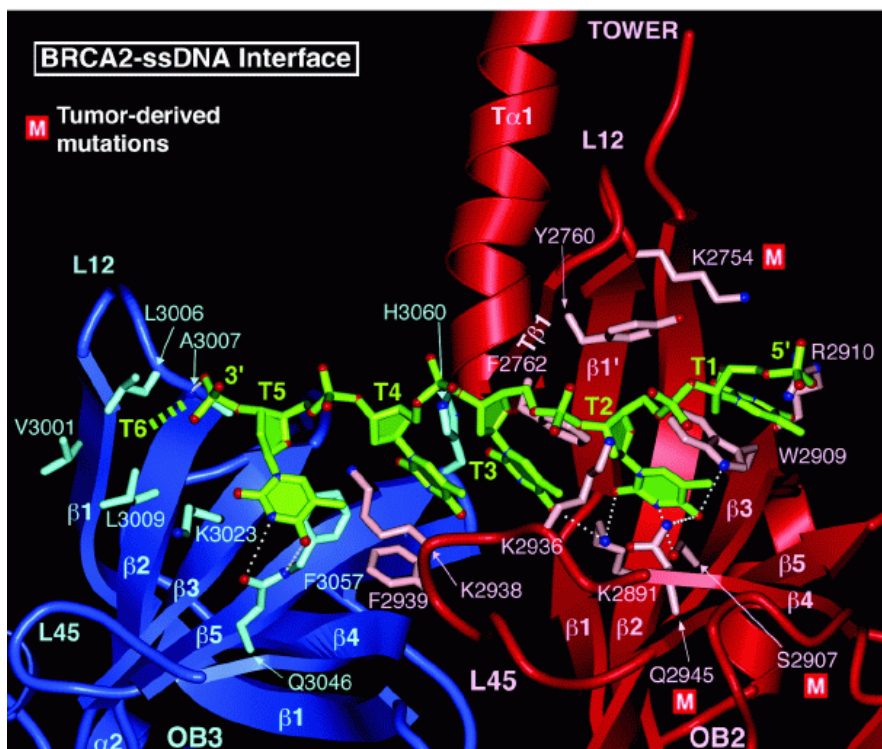
Nikola Pavletich

Investigator in Cellular Biochemistry and Biophysics Program at Memorial Sloan-Kettering Cancer Center

Nikola Pavletich is trying to understand how the normal controls on cell growth and proliferation are disrupted in cancerous cells. To do this, he looks at differences between regulatory proteins in normal and neoplastic cells; particular proteins of interest have included cell cycle regulators, p53 and other tumor suppressors, and elements of the ubiquitin-regulated degradation pathway. To determine these structures, Pavletich and co-workers visit several synchrotron sources, and spend several days a year at CHESS.



Nikola Pavletich



In just the last year, Pavletich and co-workers have reported two important structures: SCF complexes are a large family of proteins which regulate the ubiquitination of a variety of regulatory and signaling proteins. Ubiquitination is the means by which a cell marks unwanted proteins for destruction, and disruption of this regulation can contribute to neoplastic transformation. The structure of one of these SCF complexes [3] reveals how the proteins in the complex interact with each other, and suggests a mechanism by which the complex may act to bind ubiquitin to another protein. BRCA2 is a tumor suppressor protein; people with a mutation in the gene for this protein are at increased risk for breast cancer, due to a defect in DNA repair. The structure of BRCA2, in complex with the DSS1 protein and a piece of single-stranded DNA, was reported in a cover story in *Science* [4]. The structure (shown in the figure) provides indications of how the proteins interact with DNA in the repair process.

References

- [1] Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. The Structure of the Potassium Channel: Molecular Basis of K^+ Conduction and Selectivity, *Science* 280, 69-77 (1998).
- [2] Dutzler, R., Campbell, E. B., Cadene, M., Chait, B. T., and MacKinnon, R. X-ray Structure of a ClC Chloride Channel at 3.0 Å Reveals the Molecular Basis of Anion Selectivity, *Nature* 415, 287-294 (2002).
- [3] Zheng, N., Schulman, B. A., Song, L., Miller, J. J., Jeffrey, P. D., Wang, P., Chu, C., Koepp, D. M., Elledge, S. J., Pagano, M., Conaway, R. C., Conaway, J. W., Harper, J. W., and Pavletich, N. P. Structure of the Cul1-Rbx1-Skp1-F-box^{Skp2} SCF Ubiquitin Ligase Complex, *Nature* 416, 703-709 (2002).
- [4] Yang, H., Jeffrey, P. D., Miller, J., Kinnucan, E., Sun, Y., Thoma, N. H., Zheng, N., Chen, P.-L., Lee, W.-H., and Pavletich, N. P. BRCA2 Function in DNA Binding and Recombination from a BRCA2-DSS1-ssDNA Structure, *Science* 297, 1837-1848 (2002).