

**I. Structure-Function Analysis of the Mechanosensitive  
Channel of Large Conductance. II. Design of Novel  
Magnetic Materials using Crystal Engineering.**

Thesis by

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*in memory of Teresa Hsu*

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## **Abstract**

The ability to crystallize and structurally characterize ion channels has made it possible to consider the molecular motions involved in gating these channels. The crystal structure of the mechanosensitive channel of large conductance from *M. tuberculosis* (Tb-MscL) has provided new opportunities to explore mechanosensitive channel function, by providing a high resolution image of the closed state of the channel. The first section of this work describes progress towards the functional characterization of the molecular motions involved in channel gating. A general background to the approaches employed here is given in Chapter 1.

In Chapter 2, sequence analysis of 35 putative MscL homologues was used to develop an optimal alignment for *E. coli* and *M. tuberculosis* MscL and to place these homologues into sequence subfamilies. Using this alignment, previously identified *E. coli* MscL mutants, which displayed severe and very severe gain of function phenotypes, were mapped onto the *M. tuberculosis* MscL sequence. Not all of the resulting *M. tuberculosis* mutants displayed a gain of function phenotype; for instance, normal phenotypes were noted for mutations at A20, the analogue of the highly sensitive G22 site in *E. coli*. A previously unnoticed intersubunit hydrogen bond in the extracellular loop region of the *M. tuberculosis* MscL crystal structure has been analyzed. Cross-linkable residues were substituted for the residues involved in the hydrogen bond, and cross-linking studies indicated that these sites are spatially close under physiological conditions. In general, mutation at these positions results in a gain of function phenotype, which provides strong

evidence for the importance of the loop region in MscL channel function. No analogue to this interesting interaction could be found in *E. coli* MscL by sequence alignment. Taken together, these results indicate that caution should be exercised in using the *M. tuberculosis* MscL crystal structure to analyze previous functional studies of *E. coli* MscL.

A novel fluorescence-based screen for bacterial mechanosensitive ion-channel activity is developed in Chapter 2. This assay is capable of clearly distinguishing the previously observed gain of function and loss of function phenotypes for the *E. coli* mechanosensitive channel of large conductance (Ec-MscL). The method modifies Molecular Probes' Live/Dead<sup>®</sup> BacLight<sup>™</sup> bacterial viability assay to monitor MscL channel activity as a function of bacterial survival from osmotic downshock.

Chapter 3 describes the random mutagenesis of the mechanosensitive channel of large conductance from *E. coli* coupled with the high-throughput functional screen developed in Chapter 2. This mutagenesis and screening has provided new insights into channel structure and function. Complementary interactions of conserved residues proposed in a computational model for gating have been evaluated, and important functional regions of the channel have been identified. Mutational analysis shows that the proposed S1 helix, despite having several highly conserved residues, can be heavily mutated without significantly altering channel function. The pattern of mutations that make MscL more difficult to gate suggests that MscL senses tension with residues located near the lipid



headgroups of the bilayer. The range of phenotypical changes seen has implications for a proposed model for the evolutionary origin of mechanosensitive channels.

Chapter 5 further investigates structure-function relationships in the mechanosensitive channel of large conductance from *M. tuberculosis*. Intracellular domains are a common regulatory motif among eukaryotic ion channels. Here, we show that the carboxyl terminal domain of the mechanosensitive channel of large conductance from *M. tuberculosis* is such a regulatory domain. A combination of structural stability, measured by circular dichroism thermal denaturation, and channel function, measured by *in vivo* channel assays, were used to characterize multiple single point mutations in both the *E. coli* MscL and Tb-MscL carboxyl terminal regions. As seen previously for other regions of the channel, this work clearly highlights differences between the two channel homologues, as the carboxyl terminal domain plays no functional role in Ec-MscL. Recent Tb-MscL gating models have ignored this region of the channel, however these studies clearly indicate that the carboxyl terminus plays a central role in channel gating and therefore should be incorporated into gating models.

The second section of this work describes attempts to develop novel molecular magnetic materials using a variety of approaches. These approaches and a background to molecular magnetism are described in Chapter 6.

In chapter 7, a new class of magnetic materials that was prepared using the guanidinium sulfonate motif and 5,5'-salendisulfonic acid is described. These materials exhibit

massive magnetic frustration. The copper-manganese material has been extensively characterized using dc magnetic analysis and displays the classic signs of magnetic frustration. Although it is difficult to quantitate the extent of frustration in this system, the copper-manganese complex seems to display significant long-range frustration. The generality of the guanidinium sulfonate motif, using 5,5'-salen disulfonic acid as a bridging sulfonate, for the formation of magnetically frustrated materials was verified by the creation of family of six different magnetically frustrated bimetallic complexes.

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