CHAPTER 6: SUMMARY, SIGNIFICANCE AND FUTURE PLANS

In this Dissertation, I presented evidence supporting:

1) That there are no residues in the transmembrane α helices that are required for the formation of functional and regulated Cx43 hemichannels.

2) That the main pore-lining helix of Cx43 hemichannels (helix C in the structure derived from cryoelectron-microscopy data) is transmembrane helix 2 (M2), whereas transmembrane helix 3 (M3) is the secondary pore-lining helix (helix B).

3) The model of the transmembrane pore of Cx43 hemichannels derived from the spectroscopic measurements presented in my Dissertation differs from other "more speculative" models, not based on solid experimental data.

4) The evidence from LRET measurements indicates that the gating of Cx43 hemichannels by PKC-mediated phosphorylation is complex: a) It results in a partial closure of the hemichannel; b) This closure requires phosphorylation of all six subunits; c) Involves movements of the C-terminal domain and significant rearrangements of pore-lining helices,

In the Dissertation, I presented several methodologies for the study of hemichannels in general and membrane proteins in particular. These include the introduction of poly-alanine helix scanning mutagenesis, the improvement of the methodology to obtain purified functional Cx43 hemichannels, the development of the methodology to generate purified hemichannels of controlled subunit composition, and the implementation and validation of the use of LRET in hemichannels.

My future plans are a natural continuation of the use of the information and methodology listed above and presented in the Dissertation, and includes:

1) The development of a complete hemichannel pore model based on more extensive LRET measurements. These will allow for the assignment of the non pore-lining helices as well as the determination of the orientation of the helices with respect to the pore. The modeling of the side-chains in the pore should provide a better understanding of the determinants of the permeability of: a) Pores formed by different connexin isoforms, and b) Pores formed by connexin mutants that cause deafness and other genetic disorders.

2) The test the model using techniques such as site-directed mutagenesis, chemical cleavage, cross-linking, and permeability assays combined with chemical modification.

3) The determination of the molecular mechanism of regulation of hemichannels by PKC-mediated phosphorylation. In particular: a) Whether the number of phosphorylated subunits produces a gradual decrease in the cross-sectional area of the pore or whether the regulation is a highly-cooperative all-or-none phenomenon, and b) How different parts of Cx43 move in response to phosphorylation, to reduce the solute permeability. Studies of molecular mechanisms of gating with sub-Angstrom resolution will be of great importance to understand the regulation of functional membrane proteins in their native environment. The importance of understanding how phosphorylation by PKC gates Cx43 hemichannels is underscored by the role of the opening of hemichannels by dephosphorylation under ischemic conditions. This hemichannel opening contributes to the ischemic cell damage, as detailed in the Dissertation.