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Funding Source: MARC/IMSD - NIH-funded Maximizing Access to Research Careers/ Initiative for Maximizing Student Diversity

## **Optimizing the Intermedilysin(ILY)- hCD59 Receptor System of Rapid Cell Ablation in Zebrafish**

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Targeted cell ablation is a powerful and important tool for studying cellular processes. Yet current methods are slow, requiring hours to days, making them unsuitable for studying rapid cellular events lasting seconds to minutes. The Chandrasekhar lab is testing a novel rapid cell ablation technology, hCD59-ILY, for feasibility in zebrafish. To do so, the lab generated a transgenic zebrafish line *Tg(zCREST:ZsGreen-P2A-hCD59)* that expresses the human CD59 receptor and zsGreen fluorescent protein in branchiomotor neurons. While preliminary cell culture studies demonstrated that these neurons undergo rapid cell lysis following treatment with ILY protein, the conditions for rapid cell ablation in vivo remain to be optimized. My project will test several combinations of hCD59 receptor and ILY protein concentrations to determine the optimal expression levels for efficient cell ablation in zebrafish embryos.

*Tg(zCREST:ZsGreen-P2A-hCD59)* zebrafish will be set up for breeding and embryos will be collected for treatment. The mRNA encoding the hCD59 receptor will be injected into the yolk syncytial layer approximately 3 hours post-fertilization then incubated in intermedilysin for 12 hours. The concentrations are as follows: ILY doses will be 4, 20, 40 µg/mL at constant hCD59 mRNA level. Several hCD59 mRNA doses (0.5, 1, and 2 ng mRNA per embryo) will be tested. The ILY-treated embryos will be examined to determine which combination produces the highest percentage of embryos with necrotic region formation. Western Blots will also be performed on select embryos from each treatment to verify the level of hCD59 expression. It is expected that an optimal combination of hCD59 receptor and ILY protein will be found that causes maximal tissue lysis, making such a combination both efficient and effective. These findings can be used for neuroscience and developmental biology research, especially in our own lab.