

Optimizing the Intermedilysin (ILY) - hCD59 Receptor System of Targeted Cell Ablation in Zebrafish

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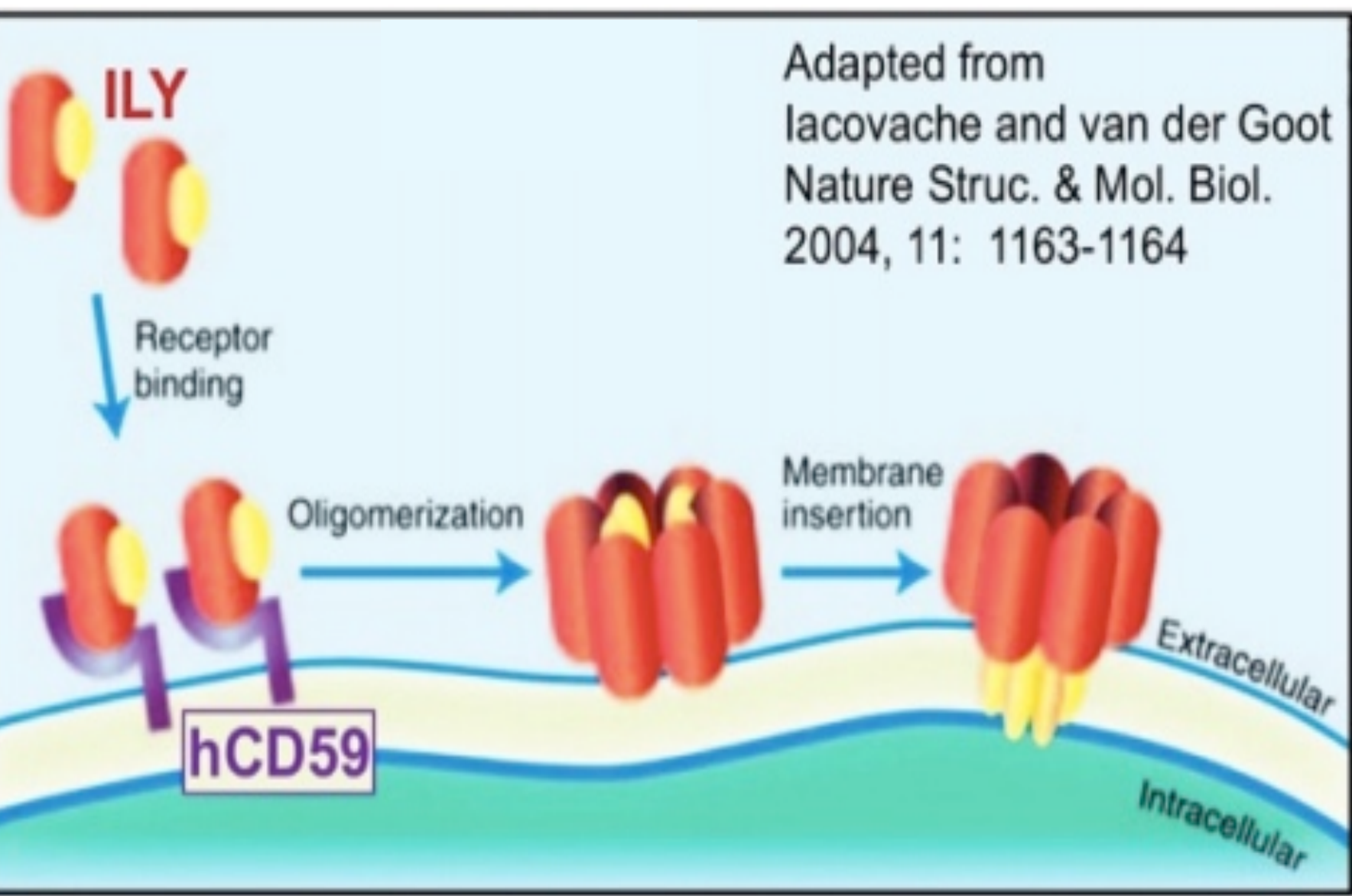
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Abstract

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:membraneRFP)* 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 efficient and effective. These findings can be used for neuroscience and developmental biology research, especially in our own lab.

Background and Hypothesis

Targeted cell ablation is the process of destroying specific cells in an organism to be able to study cellular function, lineage relationships, development, and tissue. The Chandrasekhar lab is testing a novel cell ablation technology, called hCD59-ILY, for feasibility with zebrafish.



Adapted from Iacovache and van der Goot Nature Struc. & Mol. Biol. 2004, 11: 1163-1164

Intermedilysin (ILY) is a protein that binds with the hCD59 receptor and causes pore formation in the cell membrane, ultimately causing lysis. Conditions for rapid cell ablation in vivo remain to be optimized. Among other conditions, relative concentrations of hCD59 and ILY may influence ablation efficiency. This project will look at relative concentrations of hCD59 and ILY to find a combination that will cause maximal tissue lysis in necrotic regions.

Hypothesis: A specific combination of hCD59 mRNA and ILY concentrations will be found that is both efficient and effective, causing maximal rapid cell lysis.

Obtaining Zebrafish Embryos for Experiments



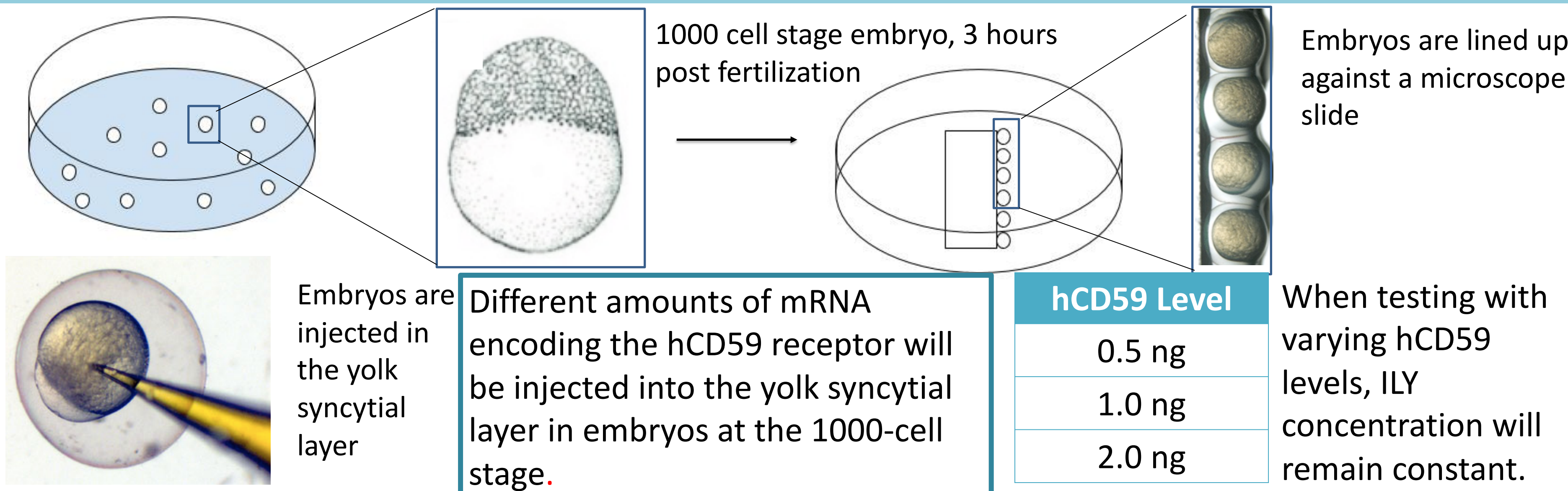
Tg(zCREST:membraneRFP) adult zebrafish will be set up for breeding overnight, and approximately 18 hours later, I will collect the embryos for hCD59 expression and ILY treatment.



Acknowledgments

I would like to thank Moe Baccam and all lab members, present and past, who have taken fantastic zebrafish care. I would especially like to thank Sam and Leon Cheng for teaching me and helping me this year. This project is funded by NIH grant R03 HD098555 and MARC-IMSD Summer Fellows.

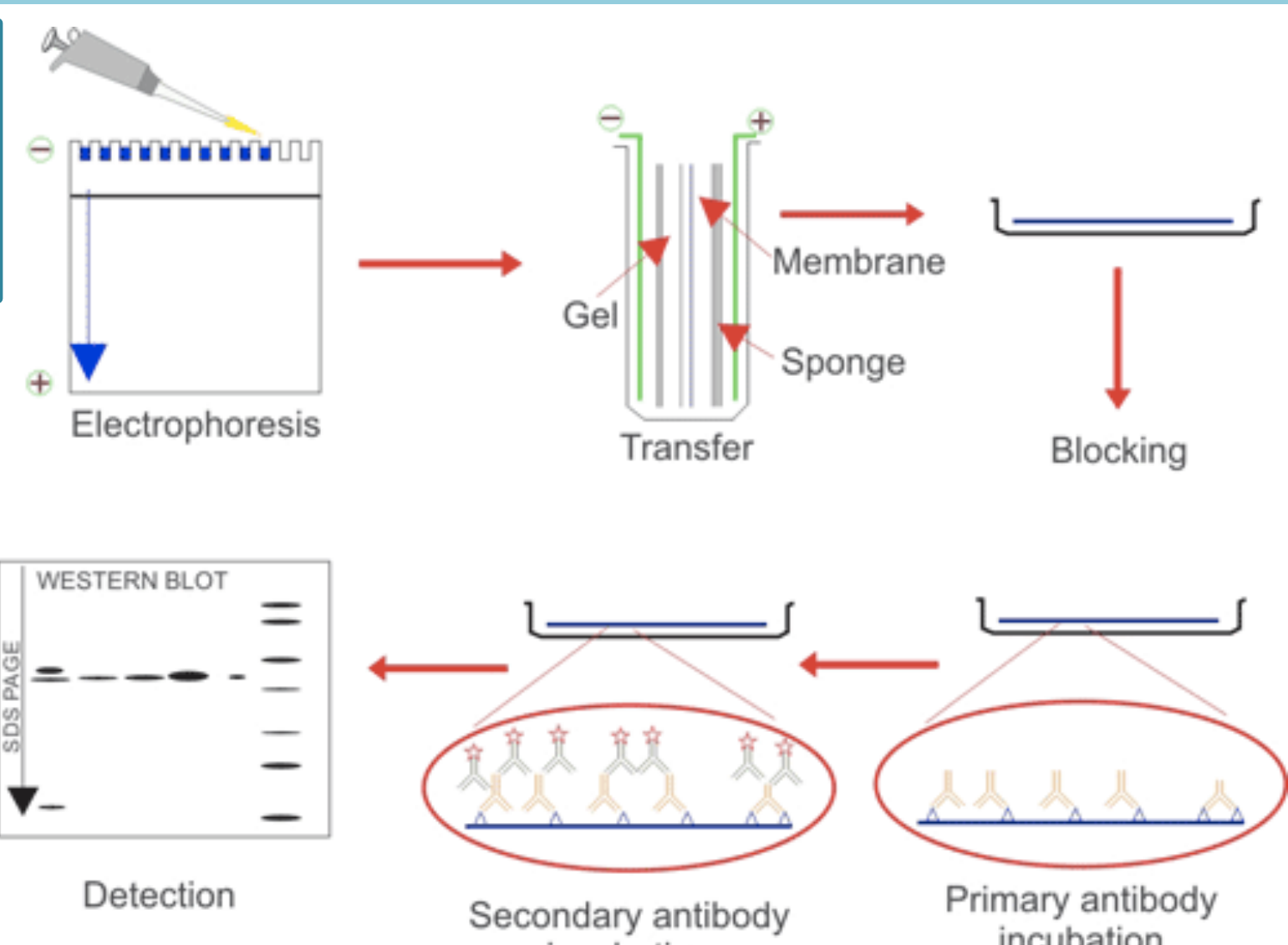
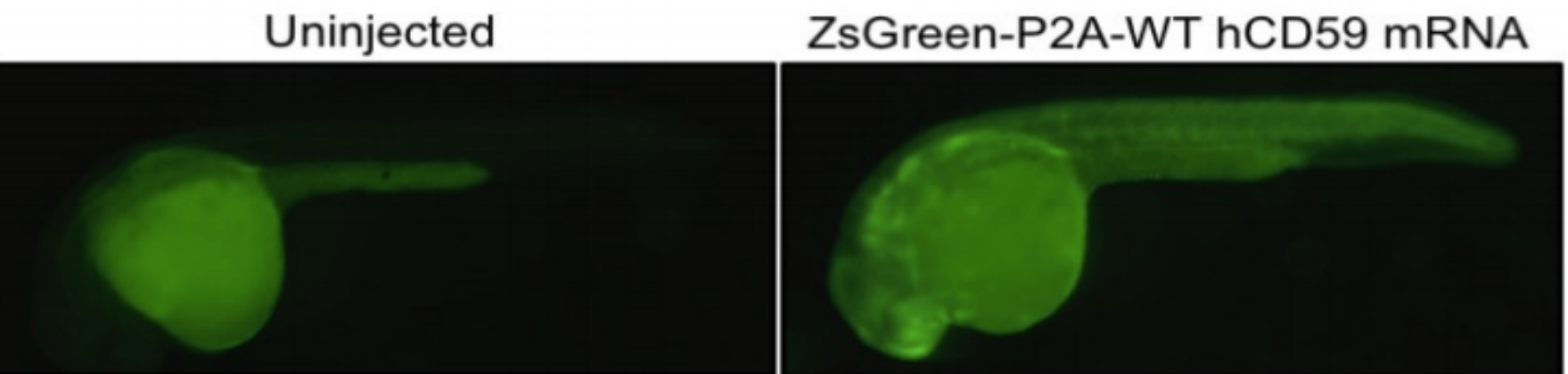
Expression of hCD59 Receptor In Zebrafish Embryos



Measurement of hCD59 Expression Level with Western Blots

A Western Blot will be conducted to confirm the expression of hCD59 and to measure the level of hCD59 protein as the concentrations of mRNA are increased.

Approximately 5 embryos from each treatment group will be used to prepare samples for analysis. In preliminary work, *Tg(zCREST:ZsGreen-P2A-hCD59)* embryos expressed *ZsGreen* when injected with hCD59 mRNA to confirm hCD59 expression



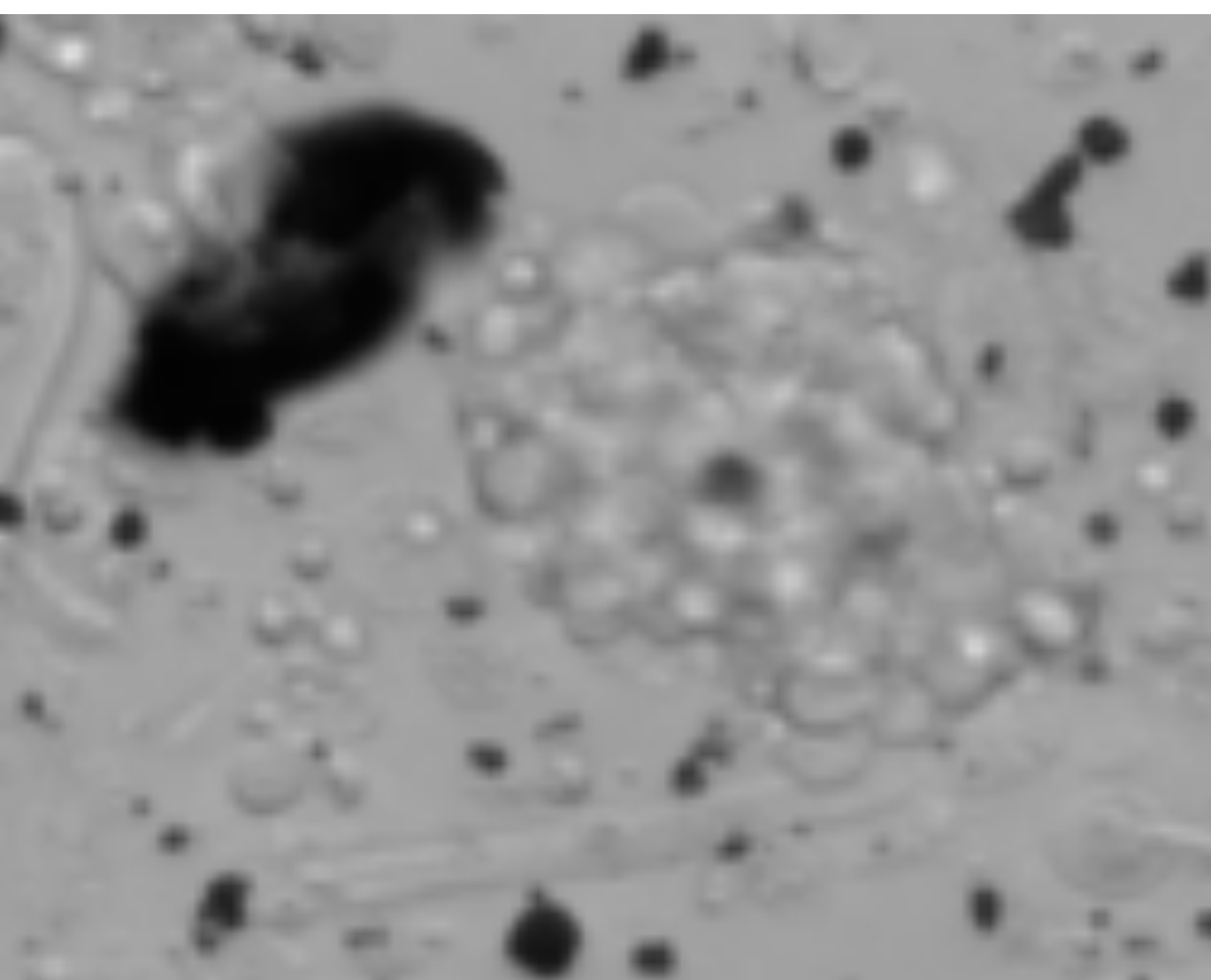
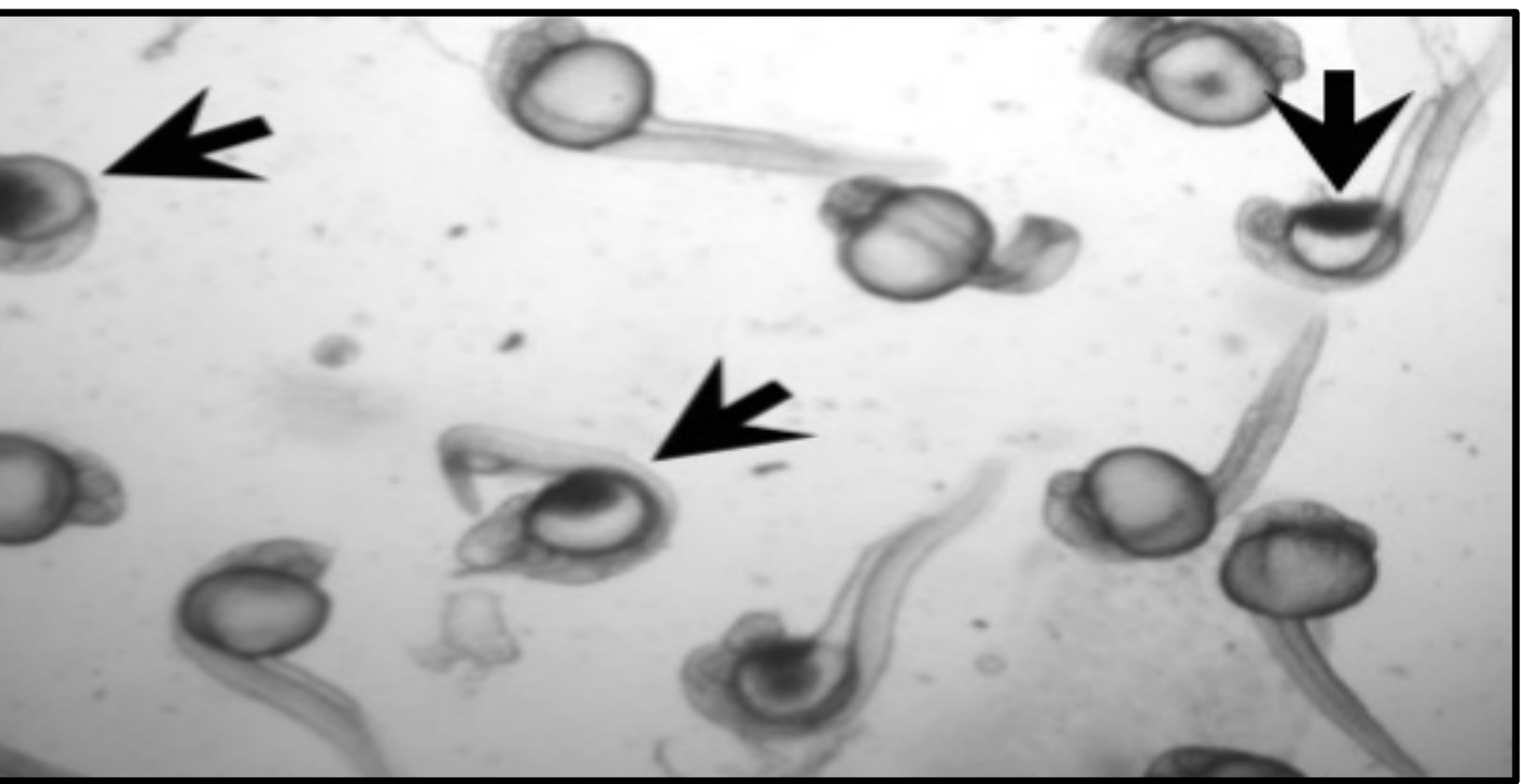
Western Blot Protocol from Cusabio Life Sciences

Cell Lysis in hCD59-Expressing Embryos Treated with ILY

No necrotic regions in uninjected control embryos



Necrotic regions form on embryos incubated in ILY



Embryo undergoes cell lysis when incubated in ILY

Once embryos have been injected, they will be incubated in ILY for 8 hours. Embryos will then be checked to see which combination forms the highest percentage of embryos with necrotic regions.

ILY Level

4.0 µg/mL

20.0 µg/mL

40.0 µg/mL

When testing with varying ILY concentrations, hCD59 mRNA concentration will remain constant.

Future Experiments

- Future work will entail actual data collection regarding this study. All information on this poster is regarding a project proposal, therefore would need to be put in action in the future.
- When this does happen, I expect to have complete involvement in my project. I will be responsible for the setting up of zebrafish to collect embryos. I will also be responsible for the injection and incubation of the embryos and checking on their progress after treatment. I will also perform the Western blot and data analysis.
- To be able to complete my duties pertaining to this study, I would need to first spend several weeks retraining myself in the injection technique and training in Western Blot technique and incubation. Once that has been completed, I will be able to partake in my project.