Investigation of an Uncharacterized *Aeromonas* Protein involved in Host Colonization

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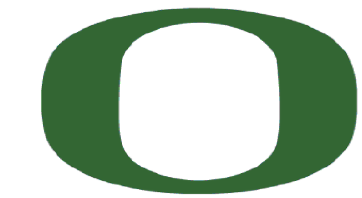

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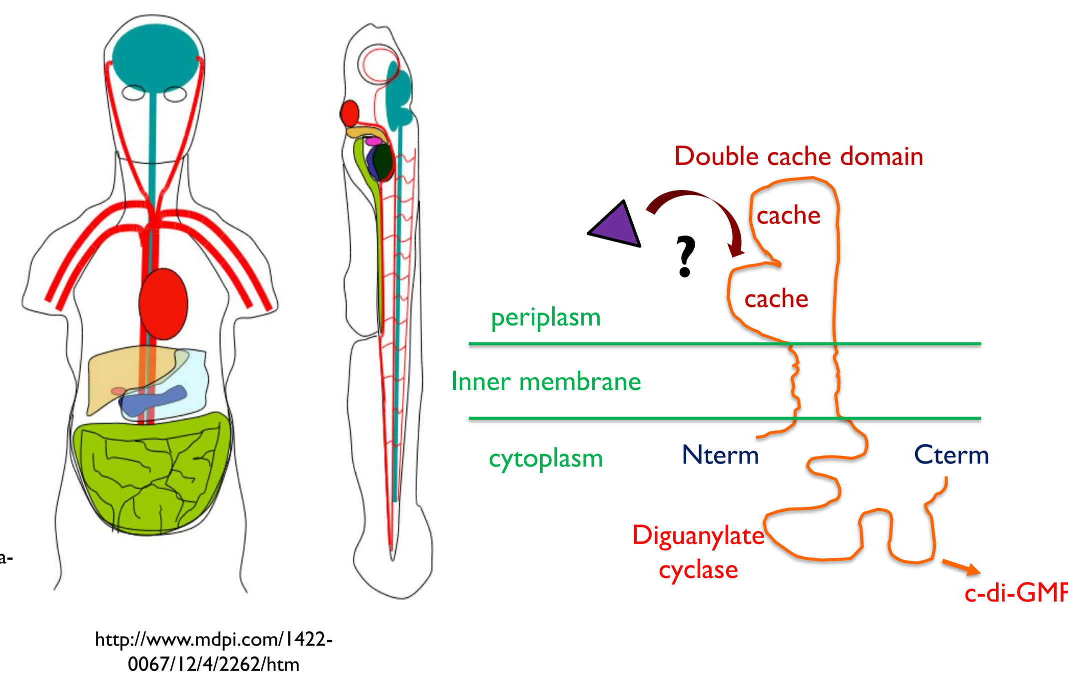
INTRODUCTION

The gut microbiota is a diverse community of microbes living in the digestive tracts of hosts like humans, animals and insects. Microbes are known to interact with host immune systems, and affect things like digestion, allergies and even development of the host.[1] However, the colonization dynamics of these gut microbes are not fully understood. In the Guillemin lab, we use a model organism based approach in order to study these host microbe interactions. The zebrafish is used as a host model organism since it shares a lot of homology with humans.[2] The bacteria this study focused on is *Aeromonas*, which is a normal resident in the zebrafish gut, and was recently discovered in the Guillemin lab to produce an uncharacterized double cache domain containing protein ZOR0001_03237 (3237). When mutated, this protein, 3237, increases the colonization of *Aeromonas* in zebrafish gut. Based on the sequence of the protein, 3237 is predicted to contain a periplasmic sensing region with tandem cache domains, a transmembrane region and a cytoplasmic diguanylate cyclase signaling domain. **This study focused on identifying ligands that bind to the periplasmic region of 3237.**

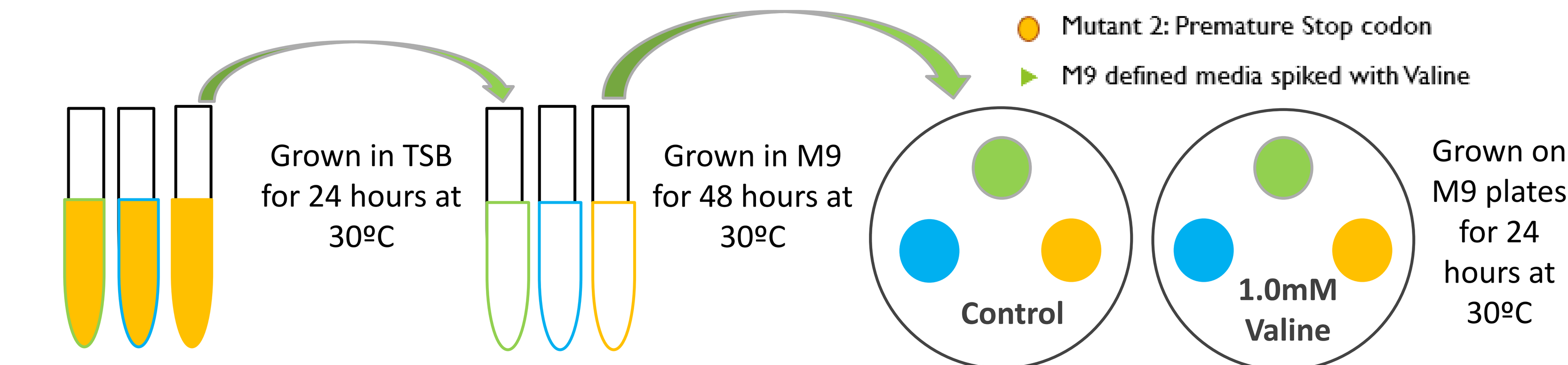
http://foodexposed.co.za/microbiome-sequenced-human-gut-bacteria-have-substantial-impact-on-health/



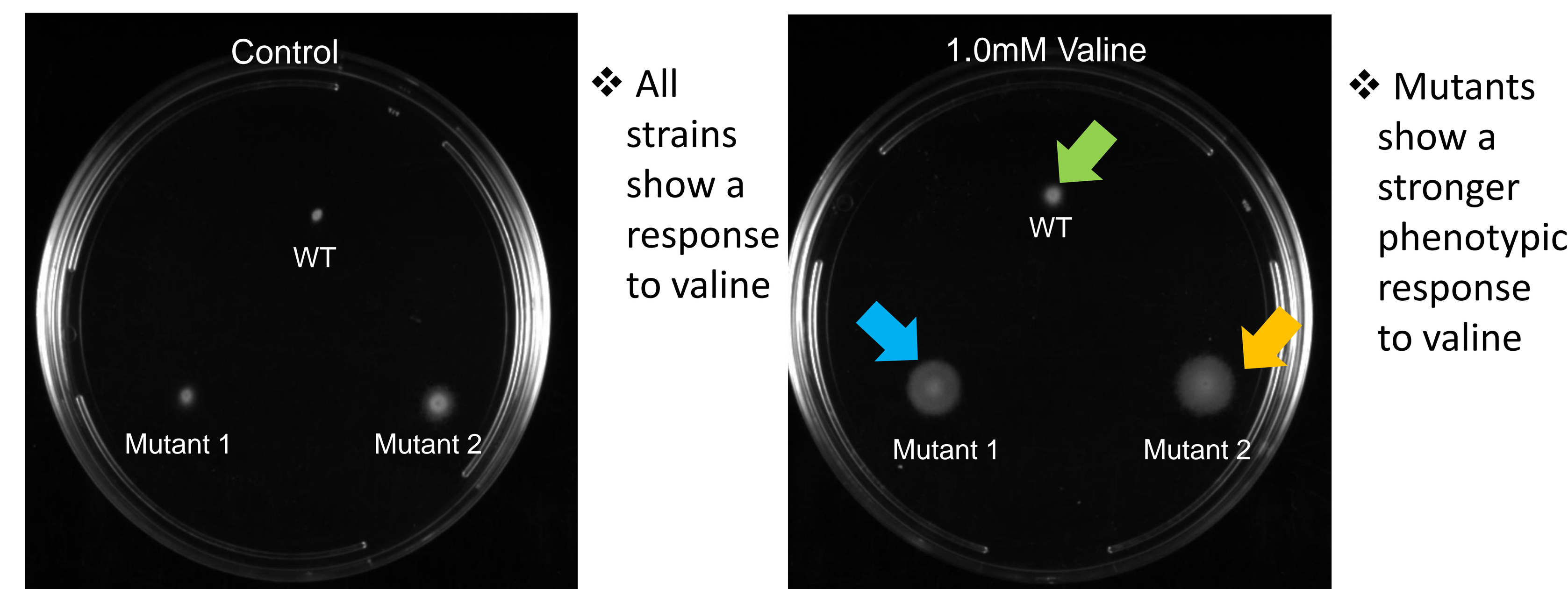
http://www.mdpi.com/1422-0067/12/4/2262/htm

SWIM PLATE ASSAY

Method



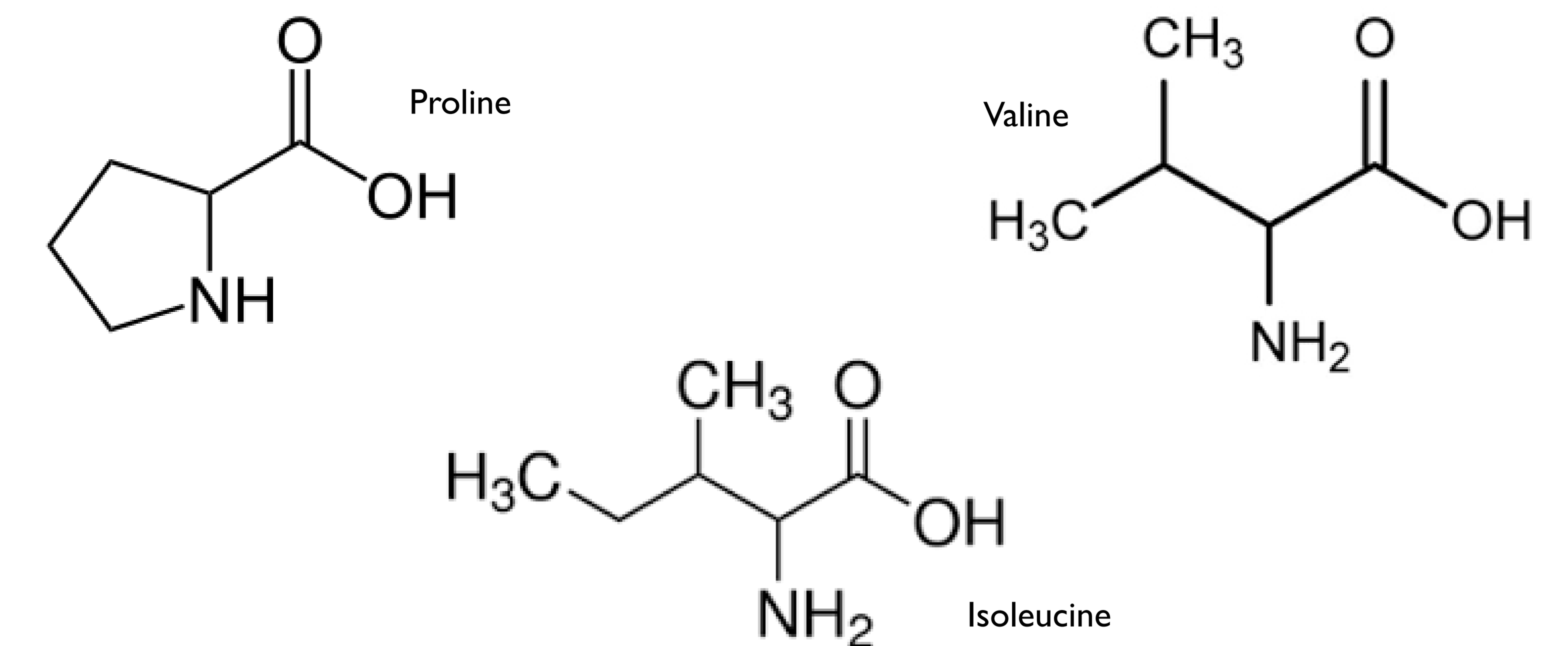
Results



- All strains show a response to valine
- Mutants show a stronger phenotypic response to valine

CONCLUSIONS

- Proline, isoleucine and valine increase the melting temperature of the protein 12-20 degrees Celsius

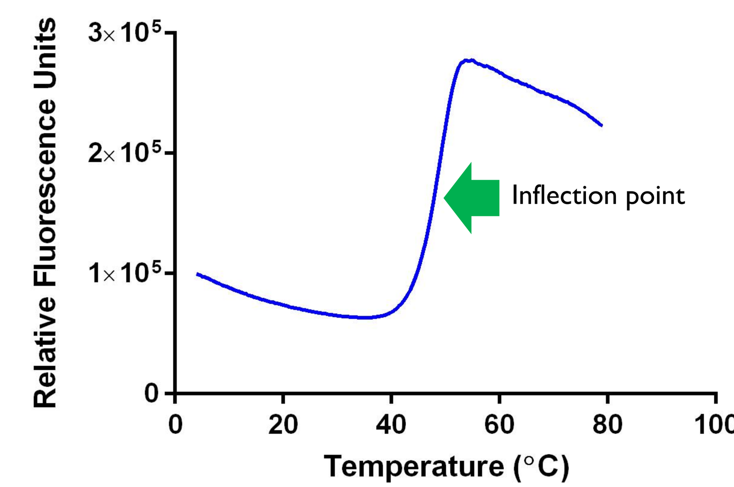


- Mutant strains of *Aeromonas* swim further with the addition of valine to defined media
- The addition of valine to the protein crystallization accelerates the formation of the cube like protein crystals from five weeks to three weeks

THERMOFLUOR ASSAY

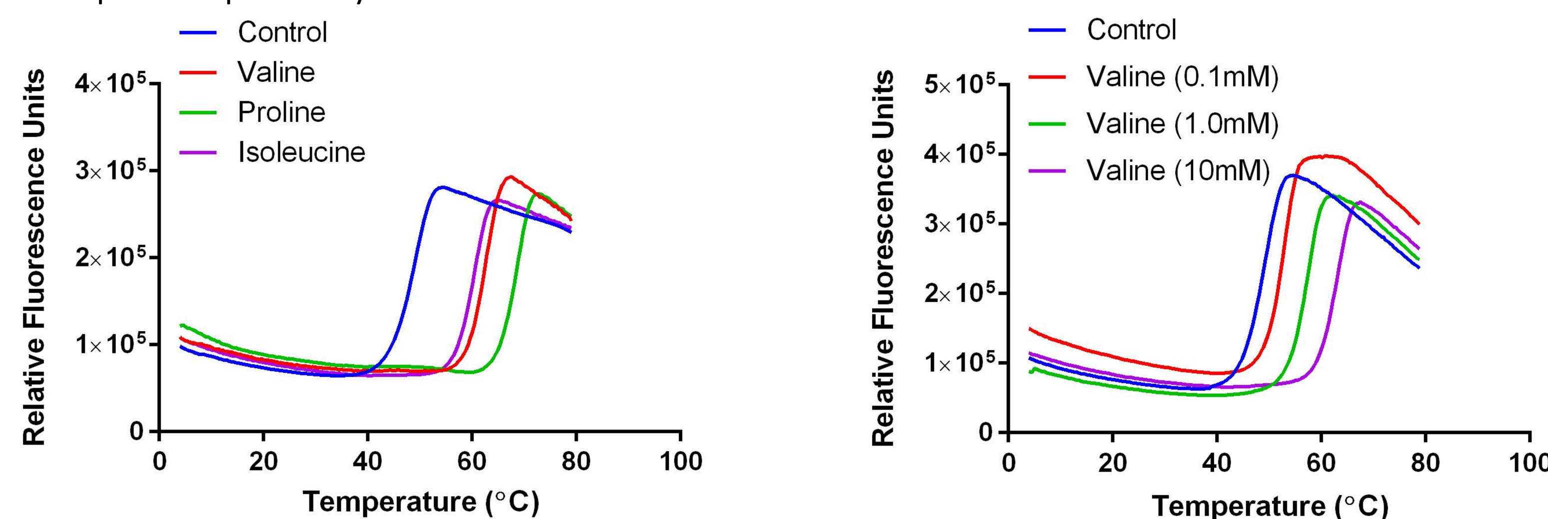
Method

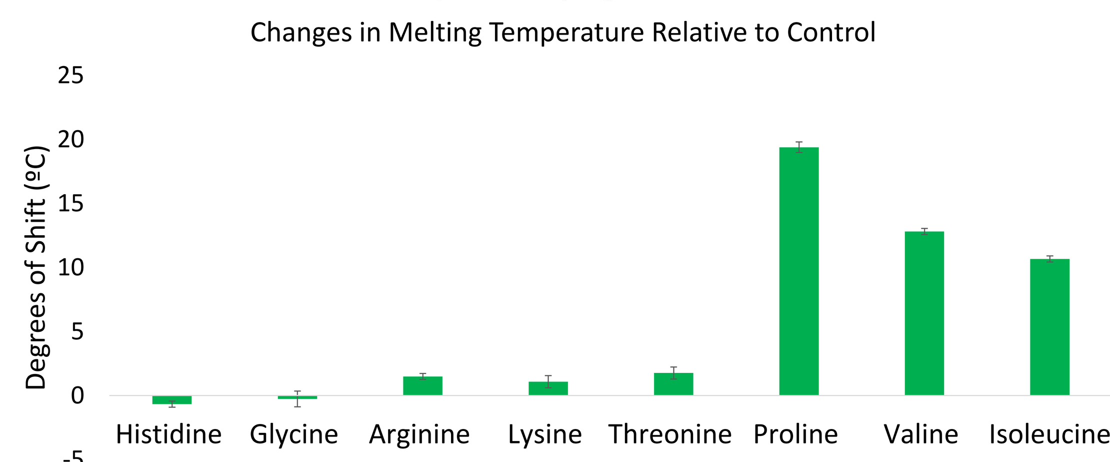
- In a qPCR machine, heat protein sample up over time to denature the protein
- Fluorescent dye SYPRO orange binds to hydrophobic regions of denatured protein
- Melting temperature is seen at the inflection point on the graph
- Bound ligands will increase the melting temperature and shift the inflection point to the right
- Screened over 200 ligands



Results

- The amino acids valine, proline and isoleucine increase the melting temperature 12-20 degrees Celsius
- The protein specifically binds to valine



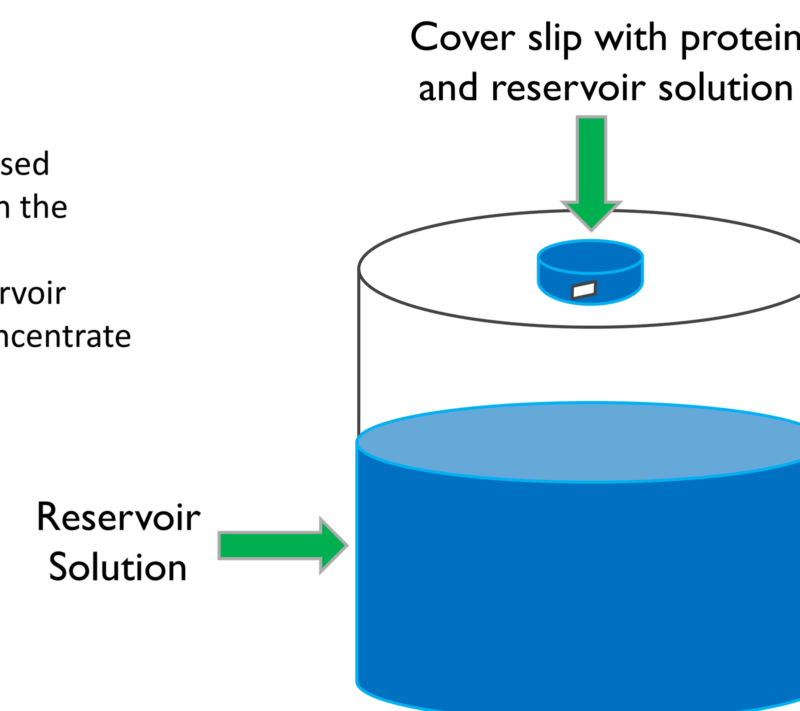


- Range of changes in melting temperature with the addition of ligands
- Only proline, valine and isoleucine show a high change from 12-20 degrees of shift

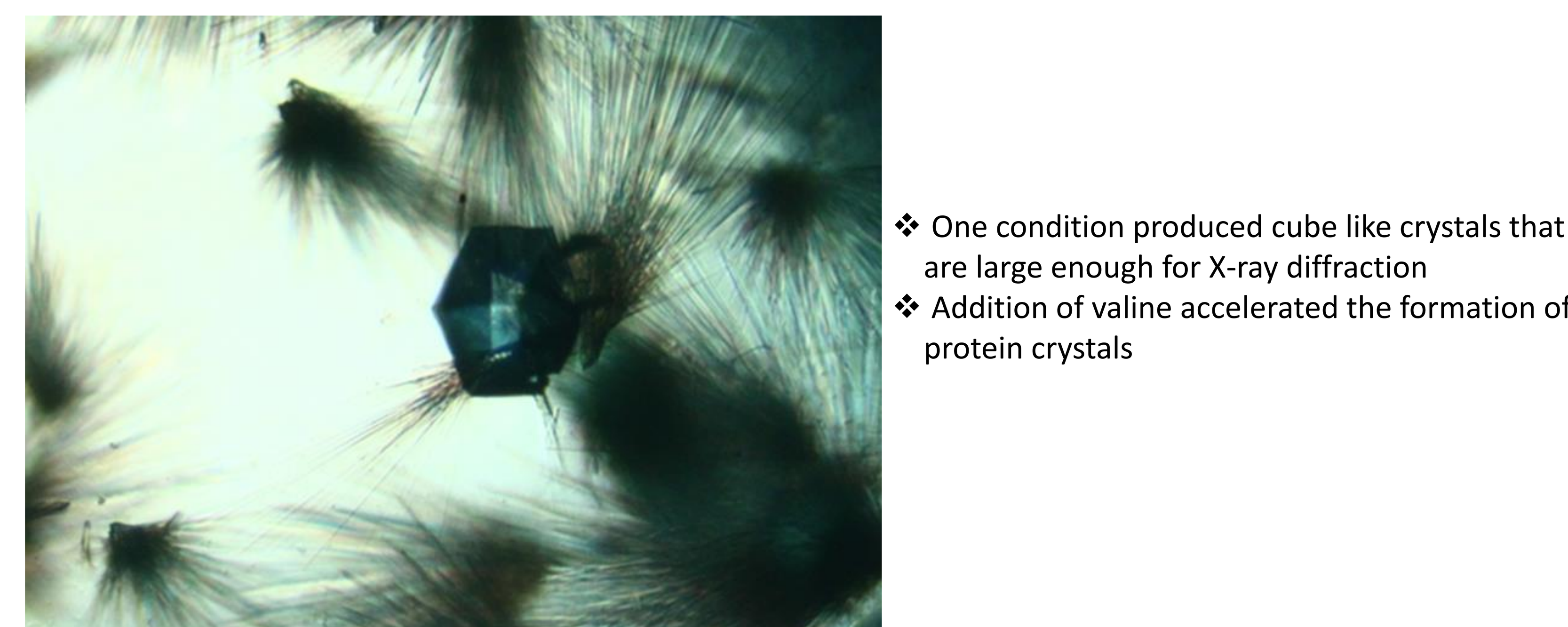
PROTEIN CRYSTALLOGRAPHY

Method

- Vapor diffusion
- Large reservoir solution
- Droplet onto a cover slip with purified protein
- Seal the cover slip onto the well to create a closed environment, and allow time for the solution in the droplet and the larger solution to equilibrate
- Cover slip droplet will diffuse towards the reservoir solution, and the protein in the droplet will concentrate and potentially form a crystal



Results



- One condition produced cube like crystals that are large enough for X-ray diffraction
- Addition of valine accelerated the formation of protein crystals

FUTURE DIRECTIONS

- Follow through with other candidate ligands: proline and isoleucine, and test them in the swim plate assay and the protein crystallography to see if they will give similar or different results as valine
- Optimize the protein crystallography to send the crystals off for X-ray diffraction to solve the structure of the protein
- Make point mutations in predicted ligand binding pocket regions to disrupt ligand binding to understand why the protein specifically binds to valine, proline and isoleucine

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CITATION

1. Quigley, E. M. (2013). Gut Bacteria in Health and Disease. *Gastroenterology & Heptology,9*(9), 560-569.
2. Barbazuk, W. B. (2000). The Syntenic Relationship of the Zebrafish and Human Genomes. *Genome Research,10*(9), 1351-1358. doi:10.1101/gr.144700