**Bioinformatics approaches to discovering antimicrobial peptides (AMPs) in the American lobster, *Homarus americanus***

**Emily Pan, Class of 2022**

 Antimicrobial peptides (AMPs) play an essential role in the nonspecific innate immune responses of lobsters. These small, cationic molecules fight off bacterial pathogens by disrupting negatively-charged bacterial membranes (Hancock et al., 2006). This antimicrobial activity is especially important during the molt cycle, in which lobsters shed their protective exoskeletons and become more susceptible to infections (Groner et al., 2020; Shields, 2019). Climate change and commercial aquaculture practices have negatively impacted the temporality of the lobster molting process in recent years (Jeffs, 2010). Therefore, understanding the structure and function of AMPs serves as important groundwork for crucial future studies related to lobster health, especially in response to anthropogenic sources of stress and disease. The knowledge gained from studying AMPs can also provide useful insight for developing novel antibiotics that are more effective against resistant bacteria (Hancock et al., 2006).

In the American lobster, AMPs are synthesized by two types of granular blood cells, or hemocytes, found in lobster circulatory fluid. So far, few lobster AMPs have been characterized and studied, so our main focus for this summer was employing various bioinformatics tools to discover new putative lobster AMPs. This process began with finding appropriate peptide sequences that could be used as queries to search for similar peptides in a hemocyte RNA transcriptome. We used an online BLAST searching tool to find possible matches for our query sequences, and analyzed the resulting peptides using a range of other online bioinformatics tools and information from literature searches. This approach helped us predict how an AMP sequence may be processed to become an active antimicrobial agent in a lobster, which then allowed us to predict the mass of each peptide that may be found in a hemolymph extract. To facilitate greater efficiency and accuracy of our bioinformatics searches, I also worked on developing an automated data-mining Python program, which helped me quickly retrieve, analyze, and organize large quantities of data.

By comparing our mass predictions for bioinformatics-derived potential AMP sequences to hemolymph mass spectral (MS) data from previous work in the Stemmler lab, we were able to identify a new putative AMP sequence with a predicted mass within 1 Da of an experimental mass. Our putative AMP sequence appears to be a type 1 crustin-like peptide, which suggests that the sequence contains both a cysteine-rich domain and the four-disulfide core whey acidic protein (WAP) domain characteristic of crustin family AMPs (Smith et al., 2008). Further analysis of MS/MS-level data and experimental work will be necessary to confirm our prediction.

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