

# Do Adhesive Mussel Plaque Proteins have Antimicrobial Properties?



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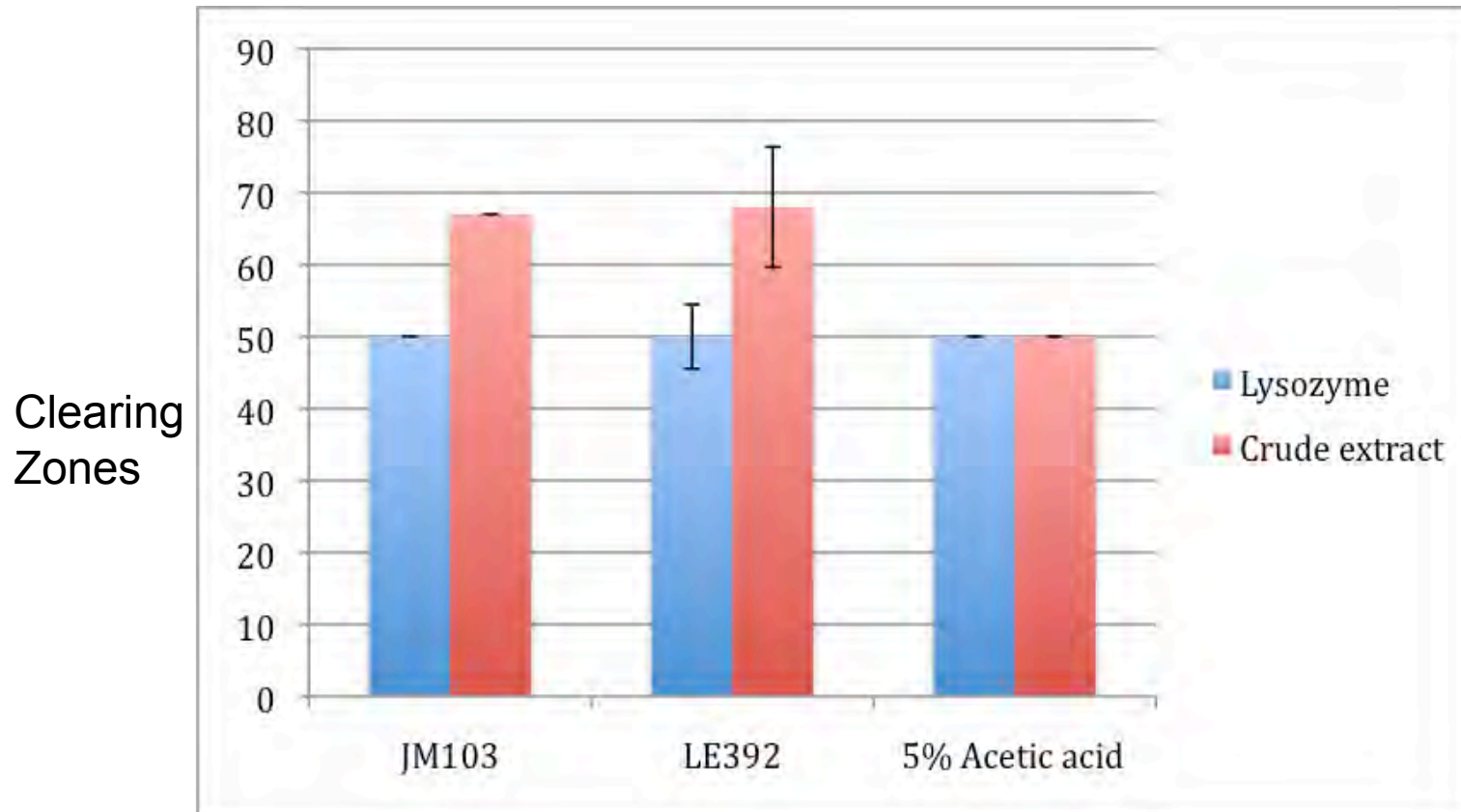
Photo: *National Geographic*

# Project goals



- To determine if proteins Mcfp-3-5 or-6, found in adhesive plaques of *Mytilus californianus*, have any antimicrobial activity.
- If the proteins do have antimicrobial activity, the biomimetic materials created from them could resist biodegradation.
- My goal is to learn and apply techniques and methods used for molecular biology and biochemistry.

# Radial diffusion assay showing inhibitory effects in arbitrary units (AU) using E. coli

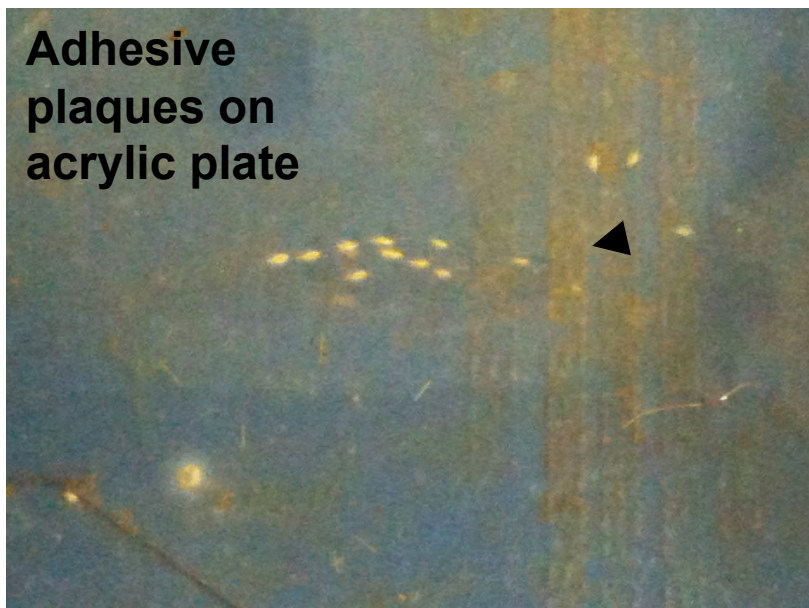


- The Mcfps have characteristics of antimicrobial peptides (AMPs).
- Making them excellent candidates for inhibiting bacterial growth.

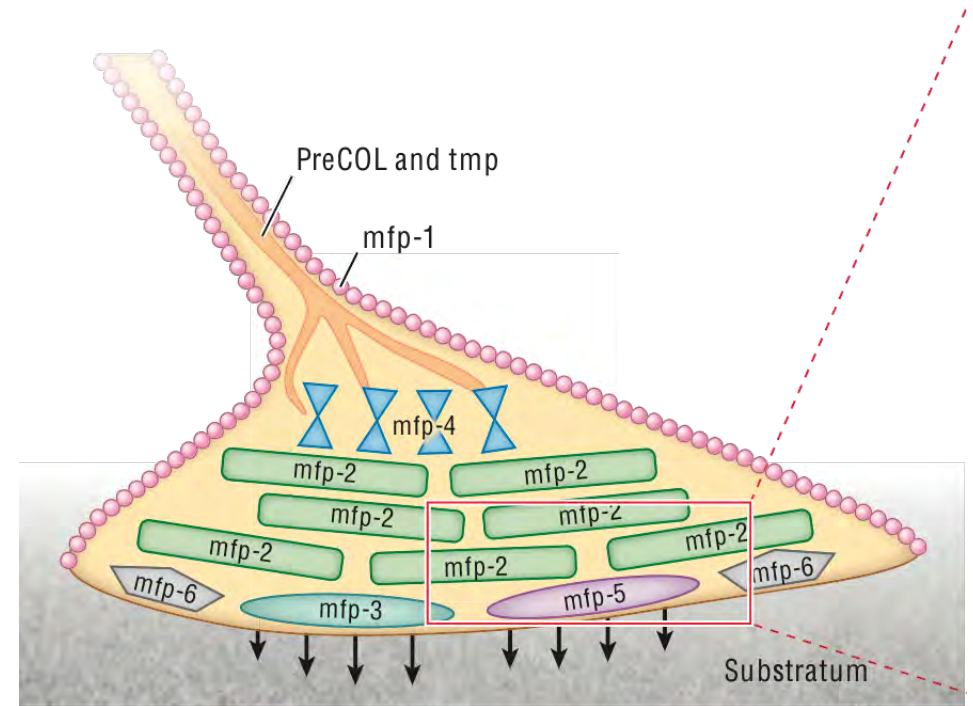
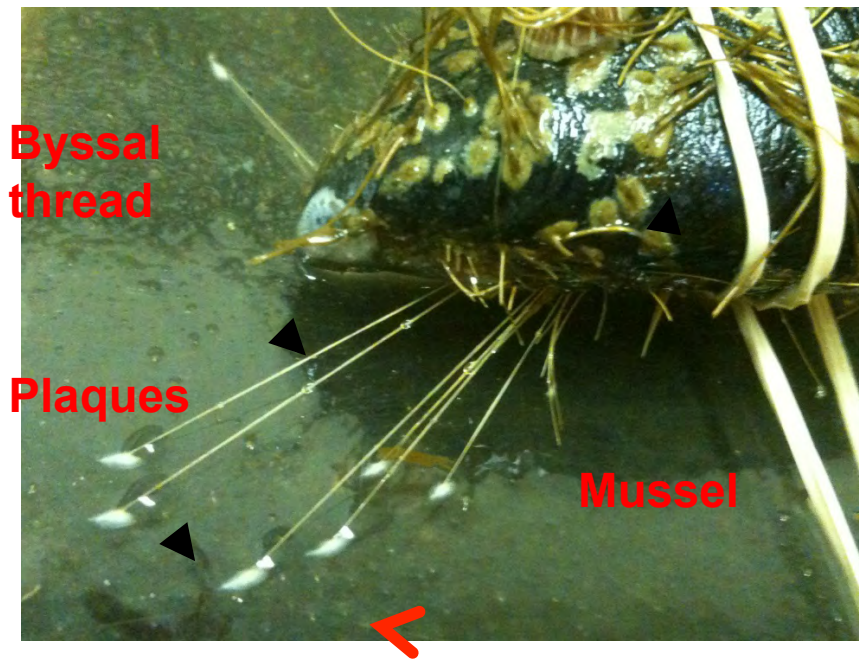
# What is a mussel's adhesive plaque?



Inside the mussel shell, there is a muscular foot which produces a thread and a plaque.



The plaques help the mussel to adhere to rocks and other substrata in its intertidal environment so it is not swept away by waves.



- This is an illustration of the mussel foot proteins (mfps) in an adhesive plaque of a marine mussel depicting the relative locations of the major proteins. Adapted from Lee et. al. 2011[31] .

# Experimental approach

Collect adhesive  
plaques  
from *Mytilus  
californianus*

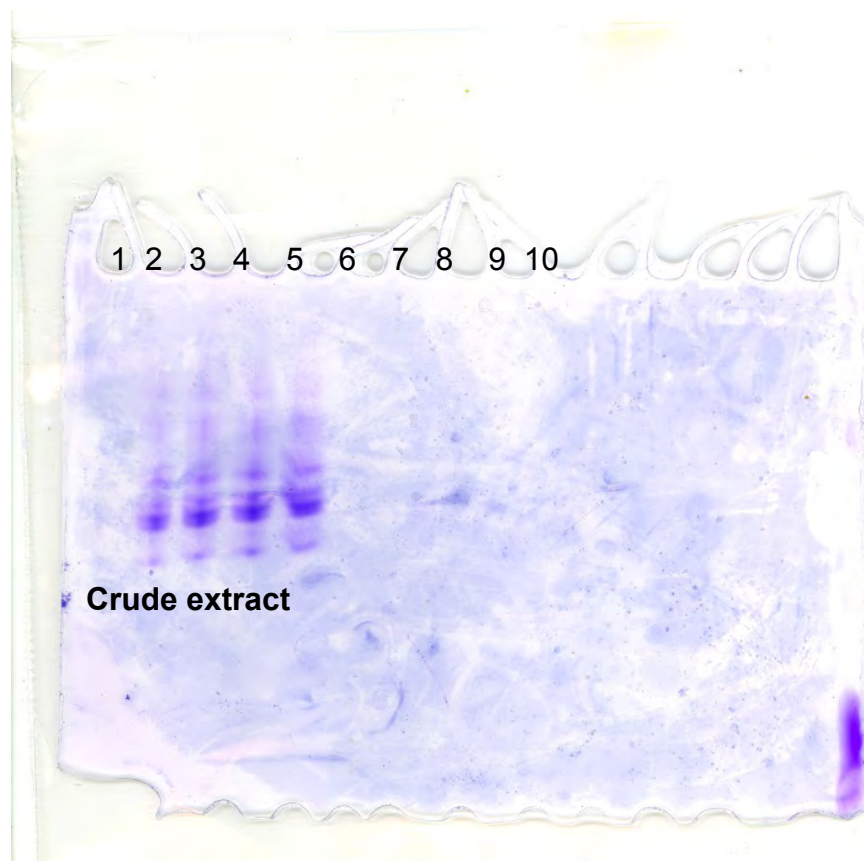
Purify Mcfps using  
Chemical and  
mechanical methods

Test Mcfps against  
*E.Coli* using Radial  
Diffusion Assay

Test Mcfps against  
other marine  
bacteria

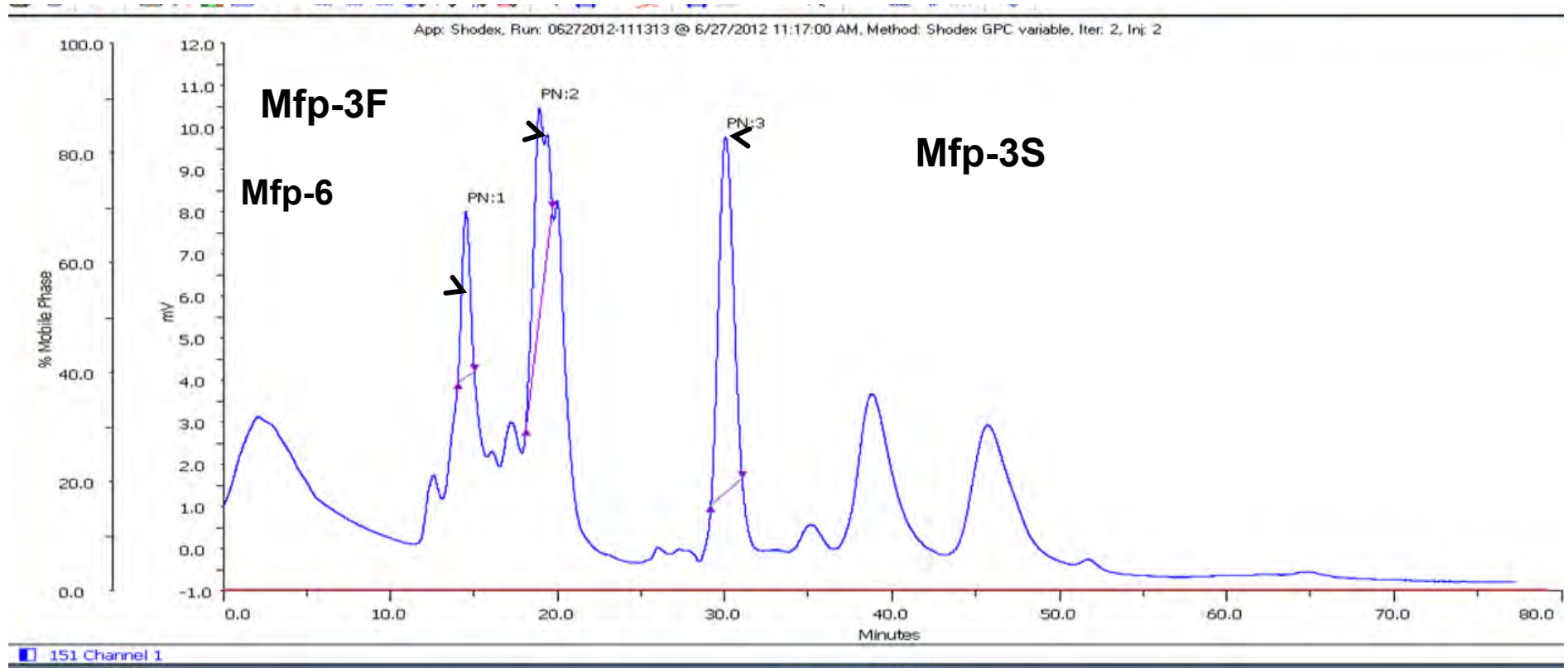
# Methods: Protein Purification

- Chemical purification: Acetic acid, urea, dialysis
- Acid/Urea Polyacrylamide Gel Electrophoresis (AU-PAGE): Both the molecular size and charge facilitate protein separation.



# Methods: Protein Purification

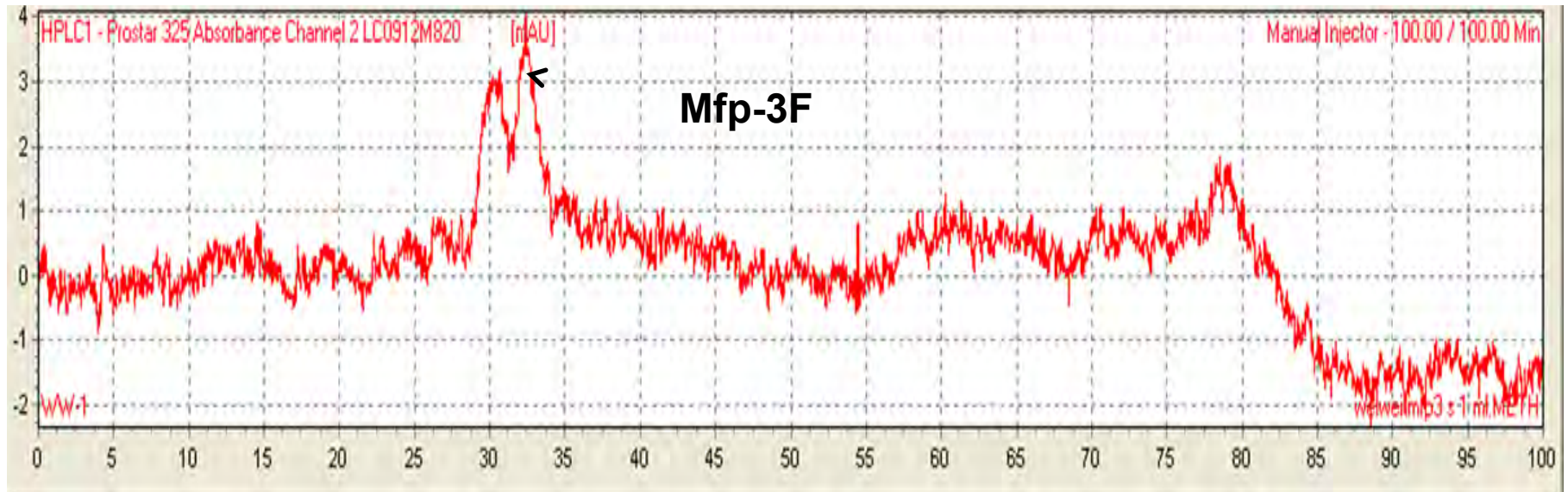
- Size Exclusion Chromatography (SEC):  
SHODEX separates proteins by size at 280nm.





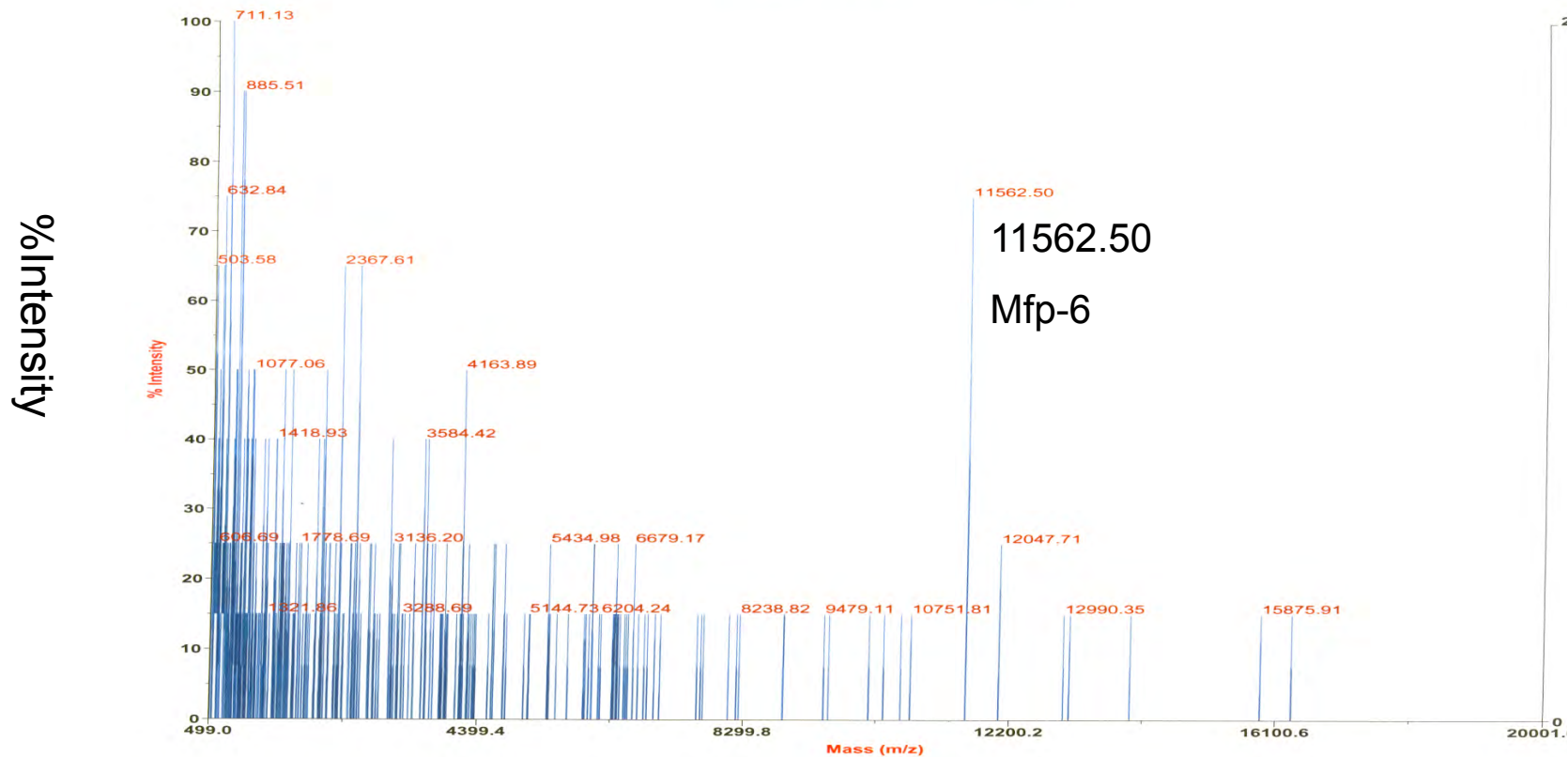
# Methods: Protein Purification

- High Performance Liquid Chromatography (HPLC): Reverse phase HPLC uses a polar mobile phase and a non-polar stationary phase.
- Retention time is longer for less polar molecules.
- Polar molecules separate out more often in the beginning stages.



# Matrix Assisted Laser Desorption and Ionization (MALDI)

- Identifies Mfps by mass



Acquired: 09:25:00, July 24, 2012

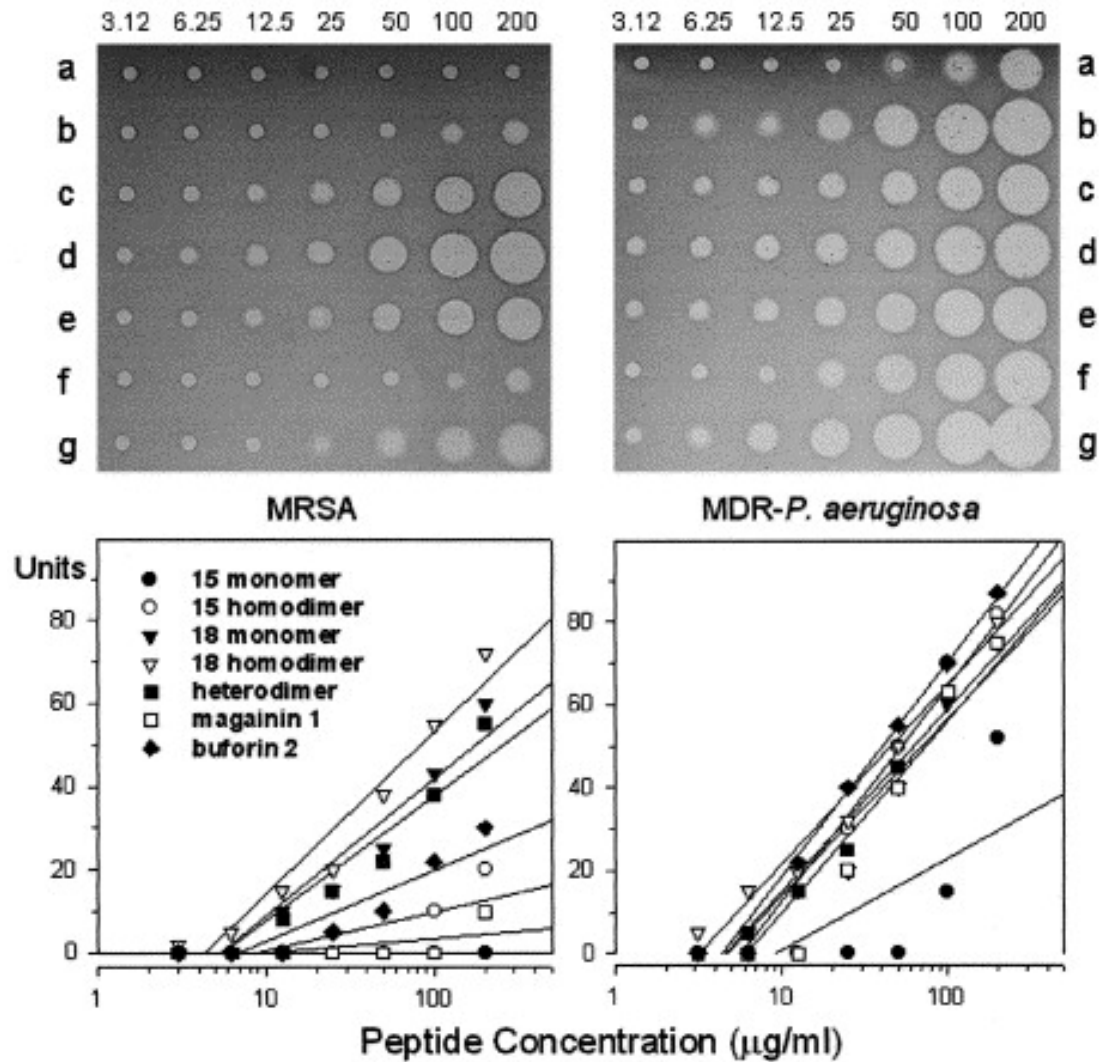
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Mass (Da)

# Methods: Determine antimicrobial effects

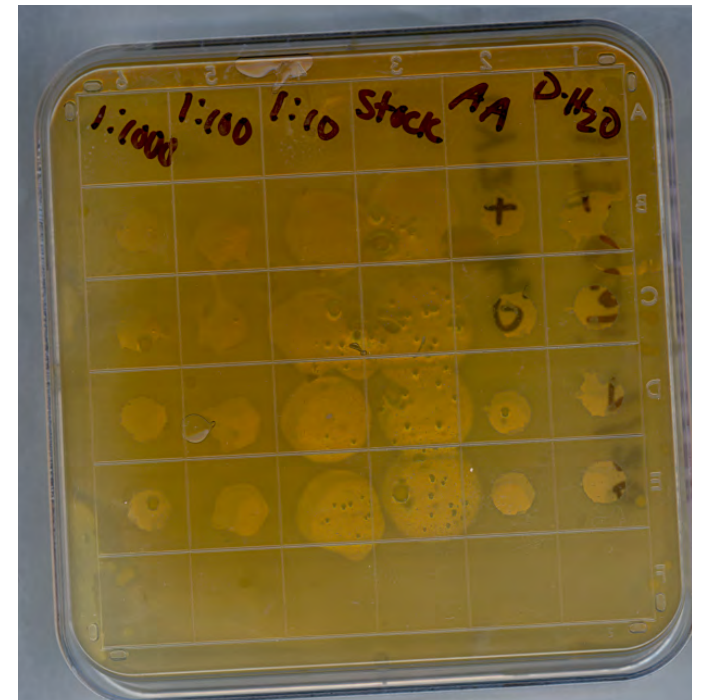
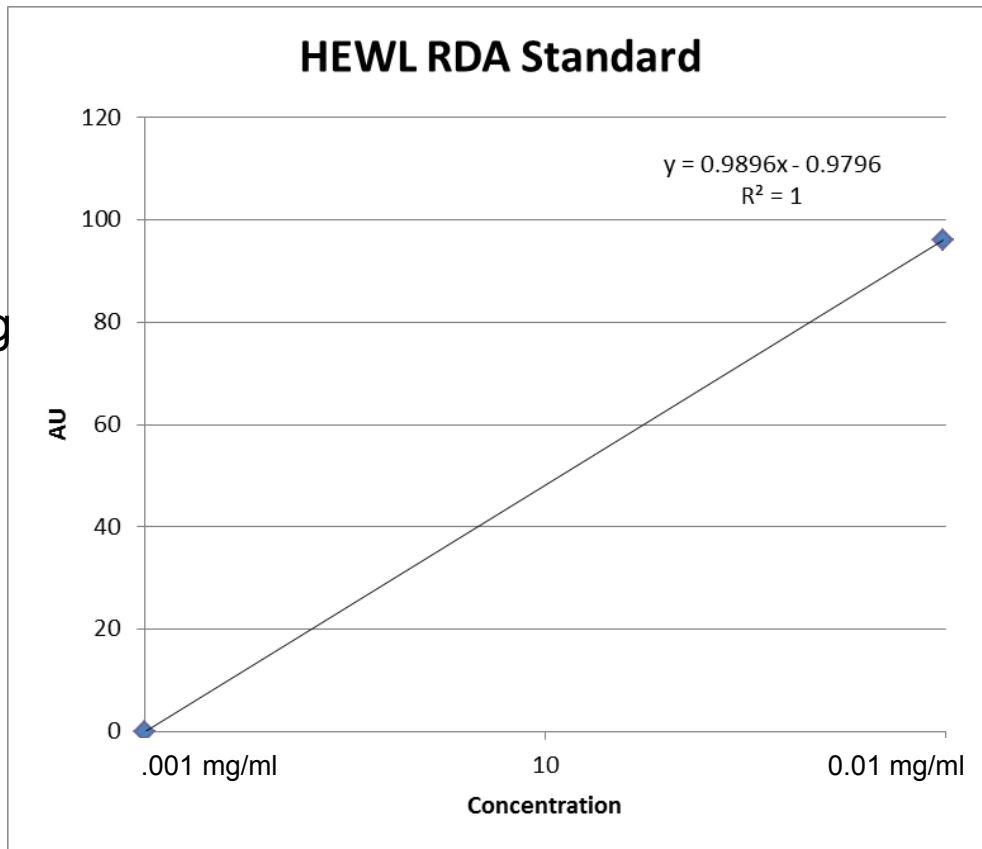
- Radial Diffusion Assay: Quantitative assay that will show whether purified Mfps have antimicrobial activity against *E. coli*.
- Lysozyme, an antimicrobial peptide (AMP) is used as a positive control.
- If the Mfps have any activity against *E. coli* using the RDA, it may show that they may act like antimicrobial peptides.

# Example of RDA using Halocidin



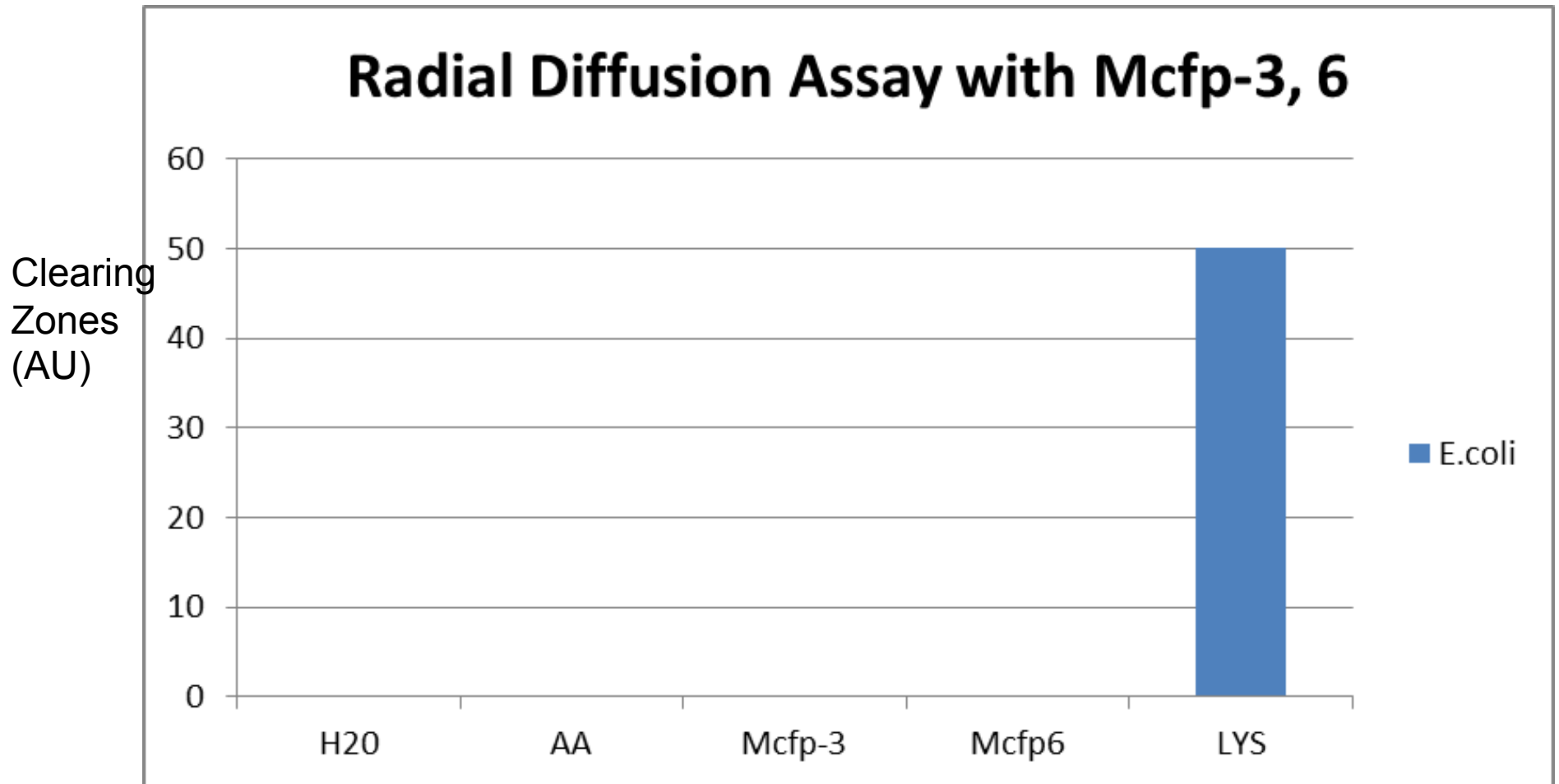
# Lysozyme Standard

Clearing  
Zones



10 AU = 1mm

# Results

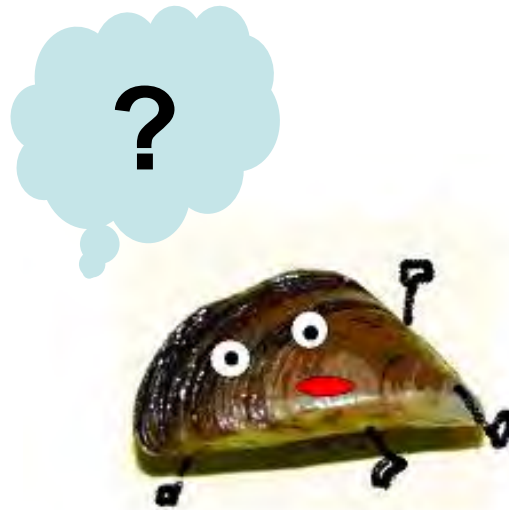


# Results

- According to the radial diffusion assay, Mfp-3, or 6 do not have antimicrobial properties against *E.coli*.
- This may be due to the Mfps not diffusing into agar well.
- An alternative microbroth dilution assay must be conducted before a final conclusion can be made.
- It is also possible that Mcfp3 and 6 may have antimicrobial action with marine bacteria.

# Future Questions

- Would Mfp-3 or -6 have antimicrobial effects on marine bacteria?
- Could the antimicrobial culprit be DOPA or a lipid?
- Is pH a factor in the antimicrobial action of the proteins?







# Acknowledgements

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