Peptide Amphiphile Micelles Aid in Diagnostics of Atherosclerosis

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SHINE
Summer High School Intensive in Next-Generation Engineering

Introduction

Atherosclerosis

- Cardiovascular disease causes over 19 million deaths/year [1].
- Atherosclerotic plaques
  - Build-up of hydroxyapatite (HA), or calcium, fat, and cholesterol [2]
  - Limits blood flow and oxygen supply from the heart to the body

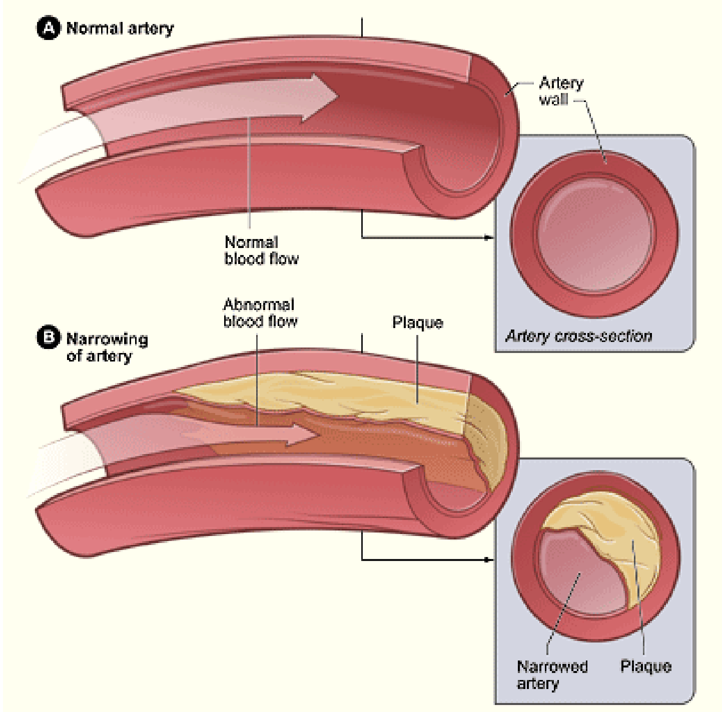

Figure 1. *Healthy vs. atherosclerotic artery. (Source: NIH)*

Objective & Impact of Mentor's Research

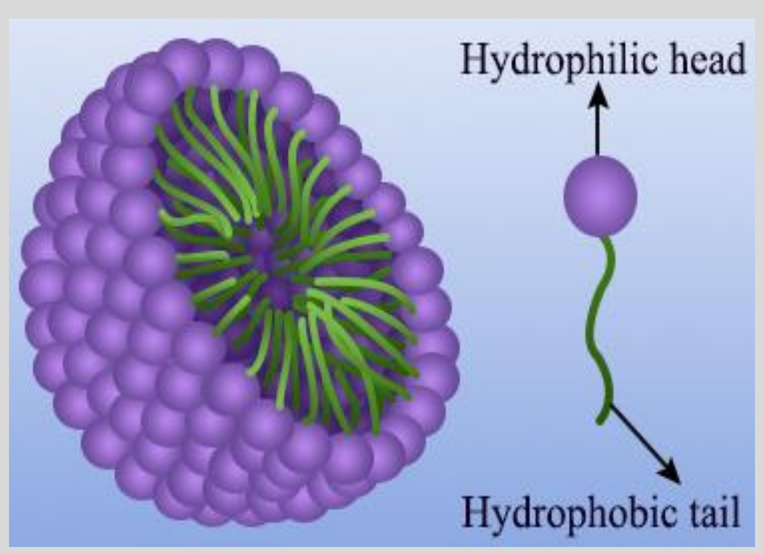

Figure 2. *Image of a self-assembling micelle (left) and peptide amphiphile (right). (Source: ScienceStruck)*

My mentor synthesizes nanoparticles called micelles that contain a peptide (HABP) that targets/binds to HA.

- Aids in early theranostics of inflamed plaques before acute cardiac event
- Fluorescent micelle with highest binding affinity to HA was used as imaging probe to locate HA embedded in plaques [2,3]

Skills Learned

Hydroxyapatite Binding Peptide (HABP) Conjugation

Hydrophobic tail was linked to HABP [SVSVGMKPSRPC] via thiol-maleimide reaction.

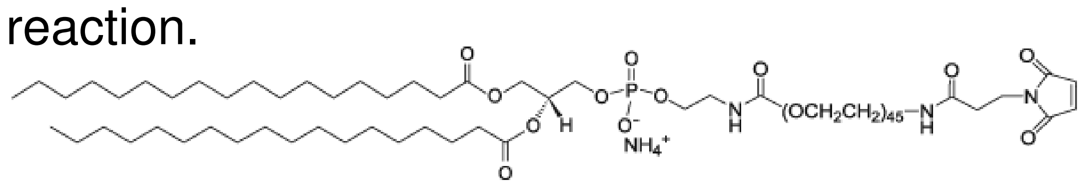

Figure 3. *Chemical structure of amphiphile DSPE-PEG-Maleimide. (Source: Avanti Polar Lipids, Inc.)*

Micelle Synthesis

Method in which three amphiphiles:

- DSPE-PEG$_{2000}$-HABP (**T**argeting peptide)
- DSPE-PEG$_{2000}$-Methoxy (**E**mpty)
- DSPE-PEG$_{2000}$-FITC (**F**luorescent)

were mixed at different molar ratios (see Fig. 4 below) to fabricate micelles exhibiting different binding affinities.

Dynamic Light Scattering (DLS)

Machine in which lasers are used to measure data such as size and zeta potential of particles.

Sample #	Micelle T:E:F Molar Ratio	Average Micelle Radius (nm)
1	90:0:10	8.8 ± 1.3
2	60:30:10	6.5 ± 0.1
3	45:45:10	5.9 ± 0.2
4	30:60:10	5.2 ± 0.2
5	0:90:10	4.9 ± 0

Figure 4. *Average micelle radius found using DLS.*

Microplate Reader

A plate reader is used to measure data such as fluorescence and absorbance.

High Performance Liquid Chromatography

Technique used to purify, separate, and identify different components in a sample. We purified the conjugated HABP sample.

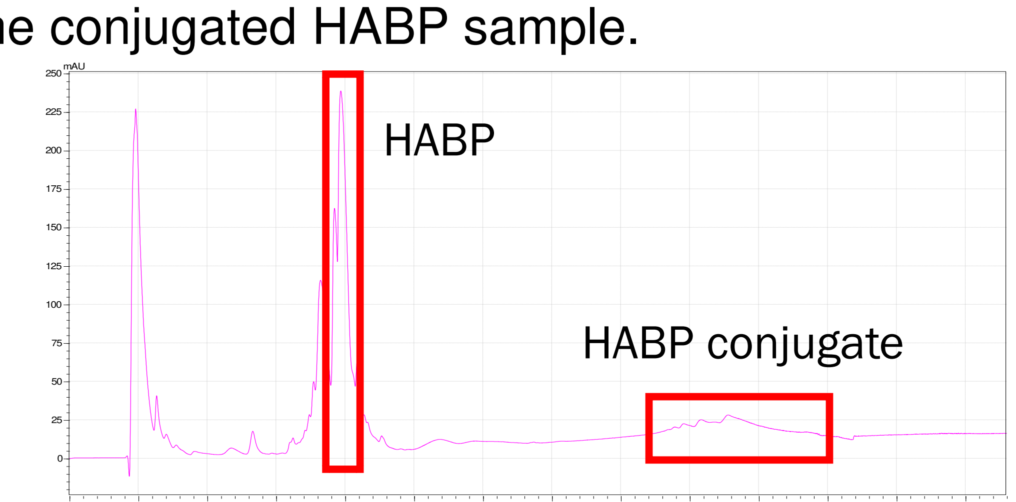

Figure 5. *HPLC graph for purifying HABP conjugate.*

How This Relates to Your STEM Coursework

Chemistry: Stoichiometric calculations, Molarity, Acids and bases, Chemical Structures, Chromatography, Titrations

Biology: Cardiovascular System, Peptides, Polymers, Binding sites

Results from HA Binding Experiments

The binding affinity of HA (powder) to HA targeting micelles were tested.

- Fluorescence intensities of five micelles were found using the Microplate Reader

$$Total\ Micelles\ - Unbound\ Micelles = Bound\ Micelles$$

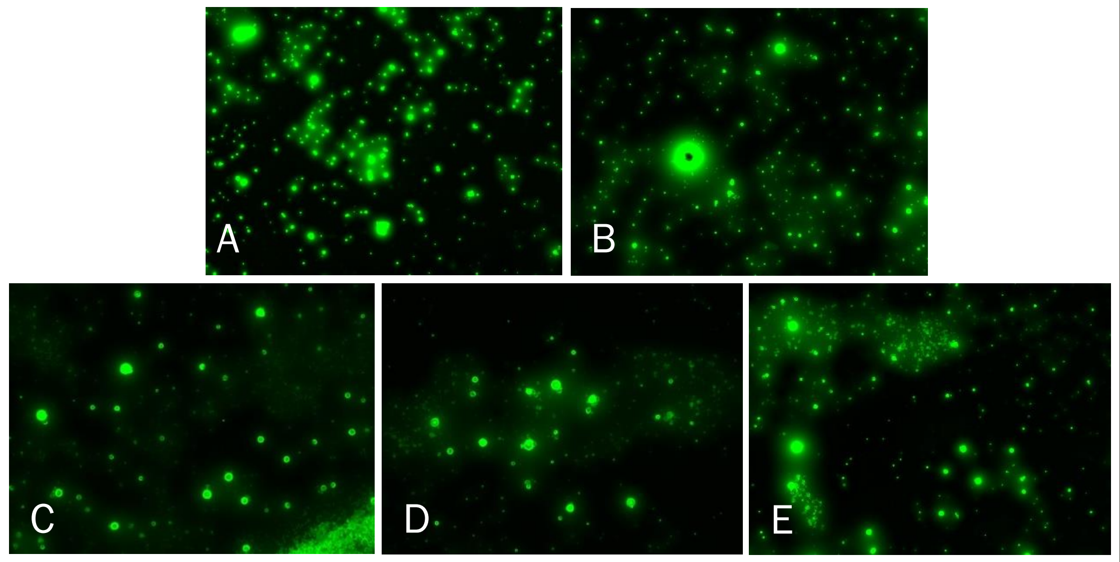

Figure 6. *TEM images of fluorescent micelles of Samples 1-5 (A-E). PC: Deborah Chin*

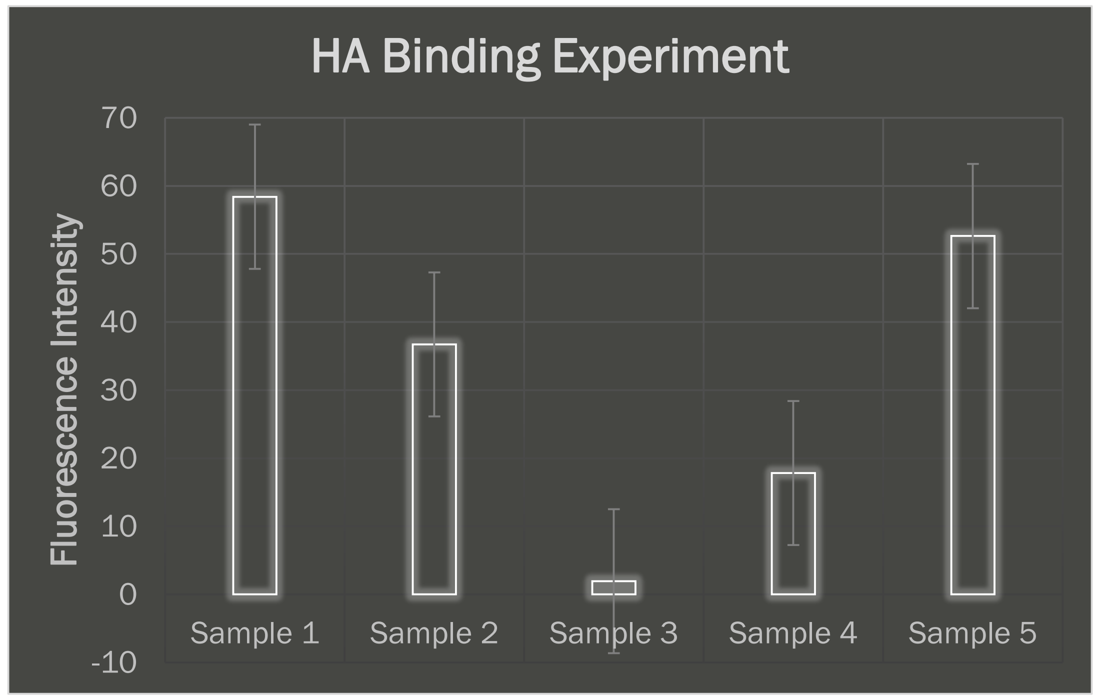

Figure 7. *Results from Binding Experiment with most comparable results. One sample = average of three replicates. Samples 1 and 5 have the highest binding affinity.*

Micelle Synthesis Stoichiometry for Desired Amphiphile Amount:

$$Volume\ of\ desired\ solvent\ (\text{mL}) \times \frac{1\ L}{1{,}000\ mL} \times \frac{100\ umol}{1\ L} \times \frac{1\ mol}{1{,}000{,}000\ umol} \times \frac{Molar\ Mass\ of\ PEG\ (g)}{1\ mol} \times \frac{1{,}000\ mg}{1\ g} = Mass\ of\ amphiphile\ needed\ (mg)$$

Next Steps for You OR Advice for Future SHINE Students

SHINE has given me so much insight on why I want to pursue Biomedical Engineering and to eventually develop therapeutics for diseases.

Advice for future SHINE students:

- Read the scholarly articles. It helps you understand your research.
- Ask questions!
- Talk to others! Meet new people who will not only help you now, but in the future.

References

[1] Naghavi, M., Libby, P., Falk, E., Casscells, S. W., Litovsky, S., Rumberger, J., ... Willerson, J. T. (2003).
[2] Lee, J. S., Morrisett, J. D., Tung, C. (2012).
[3] Lee, J. S., Tung, C. (2011).

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