ND*nano* Summer Undergraduate Research
2017 Project Summary

1. Student name & university:

Kate Mockler, University College Dublin

2. ND faculty name & department:

Professor Basar Bilgicer, Department of Chemical and Biomolecular Engineering

3. Project title:

'Engineering Multifunctional Nanoparticles For Targeted Drug Delivery in Cancer'

4. Briefly describe new skills you acquired during your summer research:

During my summer research I attained and developed a number of valuable new skills. I learnt how to employ Solid Phase Peptide Synthesis to synthesize a lipid conjugate with the targeting ligand 'LPAM1pep'. The 'LPAM1pep' ligand was synthesized using Fmoc chemistry on a solid support using Rink amide resin.

I was also introduced to High Performance Liquid Chromatography (HPLC). High Performance Liquid Chromatography is a form of column chromatography that pumps the molecular sample in a solvent at high pressure through a column with chromatographic packing material. HPLC can separate and identify compounds that are present in any sample. We used the HPLC to purify our molecules.

I was taught how to use a Rotovap, a very useful piece of equipment in any cell or biomolecular engineering lab. A Rotovap is used to evaporate off the solvent added to a molecule. The solvents are removed by simple distillation.

The most valuable skill I learnt is how to prepare and make nanoparticles. Lipid films are first made using chloroform. Using our multifaceted synthetic nanoparticle preparation method, the liposomal components can then be mixed at desired ratios during nanoparticle preparation. Lipid rafts are formed from the lipid films through the methods of agitation and hydration. Lipid extrusion is a technique in which a lipid suspension is forced through a polycarbonate filter with a defined pore size, in our case 100nm, to yield particles of the same diameter.

I was also taught how to use the Flow Cytometry equipment. Flow Cytometry is a laser-based biophysical technology employed in cell counting. My mentor David Omstead showed me the Mass Spectrometer, and I was involved in the collection of the data from this machine.

I have also become familiar with cell handling and cell culturing. I am now confident in my abilities to grow and culture both suspension and adherent cells.

## 5. Briefly share a practical application/end use of your research:

My research focused on preparing nanoparticles that could potentially deliver drugs to Multiple Myeloma cancer cells. Multiple myeloma is the second most common type of blood cancer in the US, and it is currently incurable. LPAM1 integrin is highly expressed in Multiple Myeloma cells, and its expression correlates with poor survival. One potential treatment for this type of blood cancer is to encapsulate anticancer drugs in liposomal nanoparticles, which would target only the LPAM1 receptor on the Multiple Myeloma cancer cell. This active targeting of the LPAM1 receptor would decrease the undesirable side effects due to non-specific toxicity, that non-targeted tissue and organs experience.

6. Project Abstract:

Most cancer drugs fail in clinical studies not because they are ineffective in killing cancer cells, but because they cannot be administered in doses high enough to destroy the tumor without dangerously harming the patient. One potential solution to this problem is to encapsulate anticancer drugs in liposomal nanoparticles that selectively target only cancer cells. The conjugation of ligands to liposomes enables active targeting of the tumor through specific binding to tumor-associated receptors.

7. References for papers, posters, or presentations of your research:

- Mockler, K. (2017) 'Engineering Multifunctional Nanoparticles For Targeted Drug Delivery in Cancer' [Presentation], 072617SURPS: Summer Undergraduate Research Symposium. University Of Notre Dame. 26 July.
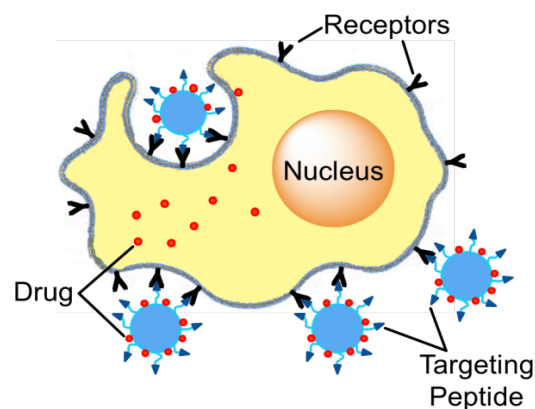
Nanoparticles are designed to deliver cytotoxic anticancer drugs, after being endocytosed by cancer cells. Here, the targeting peptide is 'LPAM1pep'.

Project summary that describes problem, project goal and your activities / results:

Most cancer drugs fail in clinical studies not because they are ineffective in killing cancer cells, but because they cannot be administered in doses high enough to destroy the tumor without dangerously harming the patient. One potential solution to this problem is to encapsulate anticancer drugs in liposomal nanoparticles that selectively target only cancer cells. The conjugation of ligands to liposomes enables active targeting of the tumor through specific binding to tumor-associated receptors. My research focused on preparing nanoparticles that could potentially deliver drugs to Multiple Myeloma cells, by targeting only these types of cancer cells. I studied the LPAM1 receptor which is expressed in Multiple Myeloma cells.

Figure 1: Molecular structure of 'LPAM1pep' with EG6 Linker drawn using chemdraw

We used a short peptide sequence as the targeting ligand, which we named 'LPAM1pep'. Unlike other lab groups, we design the nanoparticles by synthesizing the targeting ligand as a lipid conjugate before purifying the product for nanoparticle preparation. The liposomal components can then be mixed at desired ratios during nanoparticle preparation. This method provides highly reproducible results with high purity compared to other nanoparticle preparation procedures.
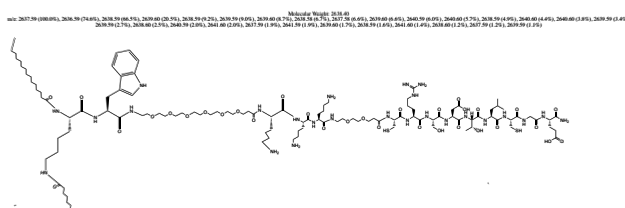
We employed solid phase peptide synthesis to synthesize a lipid conjugate with the targeting ligand 'LPAM1pep'. Studies show that LPAM1 integrin is highly expressed in multiple myeloma cells, and its expression correlates with poor survival. We then employed High Performance Liquid Chromatography to purify the molecule. Prior to nanoparticle preparation, we conducted a binding study using fluorescent labeled LPAM1pep at different concentrations to IM9 blood cancer cells. The results were analyzed using Flow Cytometry. We then prepared the nanoparticles using the same targeting ligand sequence LPAM1pep and delivered it to the cancer cells.
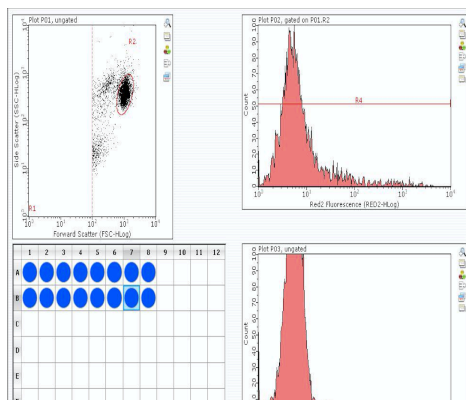

Figure 2: Flow cytometry data from cell binding assay

We also conducted a Binding & Uptake Liposome study using 'iRGD' as the targeting ligand, and H929 blood cancer cells. Lipid films were made using an EG6 linker attached to the iRGD peptide. The nanoparticles were prepared by the hydration & extrusion methods described. The cells were mixed with media and dosed with the liposomes. The samples were incubated for 3 hours, and 24 hours respectively. Trypsin was added to half of the samples in order to conduct an uptake study. The results were analyzed using Flow Cytometry.

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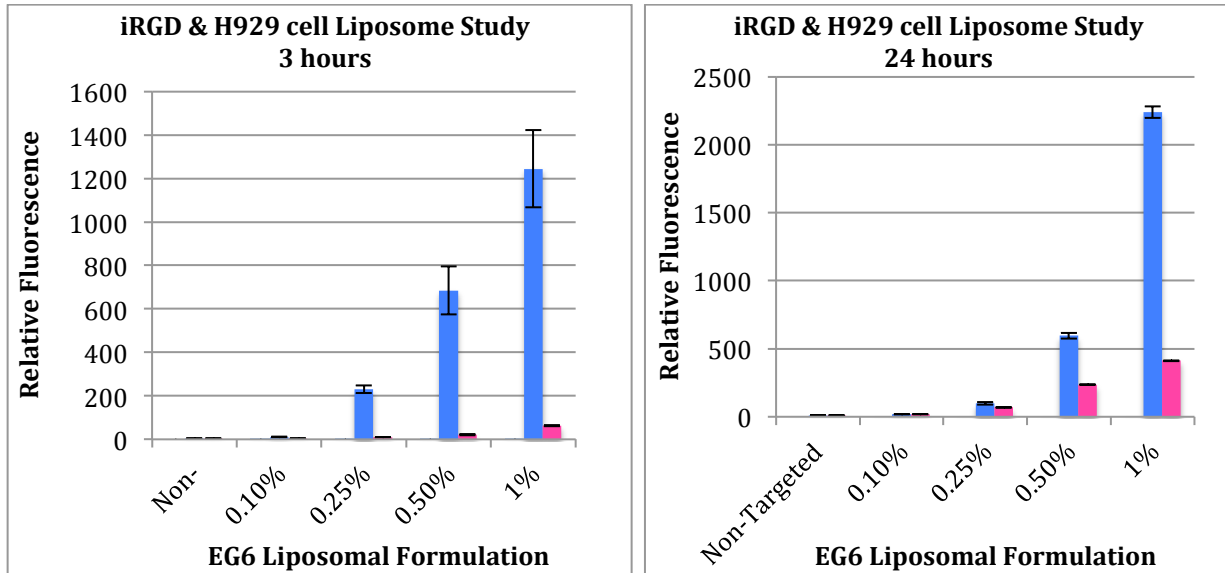
Figure 3: **C:** *Graph of iRGD-targeted Liposomes & H929 cells binding and uptake study after 3 hours.*
D: *Graph of iRGD-targeted Liposomes & H929 cells binding and uptake study after 24 hours.*

In the iRGD Liposome study, there is ~ 700% increase in liposome uptake at 1% peptide density after 24 hours vs. 3 hours. However, the cellular association for peptide densities 0.1-0.5% does not increase significantly after 24 hours, compared to after 3 hours. These results demonstrate the potential of LPAM1 and iRGD ligand-targeted liposomes to specifically target multiple myeloma cancer cells and enhance anticancer activity.

This project has motivated me to continue to work and engineer multifunctional nanoparticles in the hope that one day these targeted nanoparticles will be the most efficient form of drug delivery in cancer.