Ligand-functionalized Microbeads for Preferential Binding of Methylated and Unmethylated DNA

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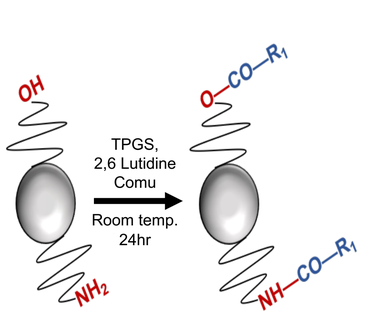
Research Motivation

Plasmid DNA (pDNA) is one of the main building blocks for vaccines and essential therapeutics for non-viral gene therapy applications. In diseases like Cancer and Alzheimer's DNA methylation plays a vital role which dictates changing the healthy settings of gene expression to a diseased pattern. Plasmids are circular double stranded DNA molecules that are different from cells' chromosomal DNA, for example they can replicate independently. To effectively use pDNA in these therapeutic applications it is necessary to efficiently isolate and purify the pDNA, which can be found in a variety of bacterial cells. Hence new approaches to enrich methylated DNA from biological samples in a rapid and cost-effective way can advance the transformative treatment and detection techniques of many diseases. Ongoing research from Dr. Rege's lab has discovered a polymer microbead (**Amikabead**) platform[1], which has shown high binding affinity to plasmid DNA molecules and demonstrates its ability for bio-manufacturing applications to be used in future biotechnology and therapeutic research. With this research, a chemically diverse lipid-modified Amikabead library will be synthesized to evaluate methylated and unmethylated plasmid DNA binding.

Methods

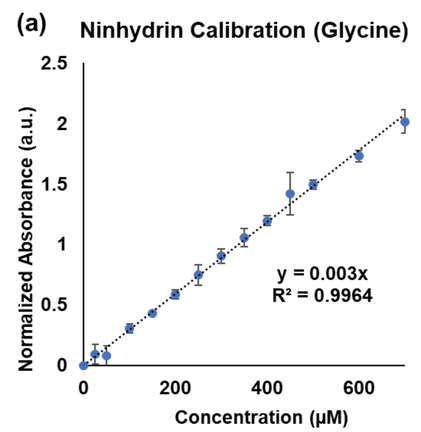


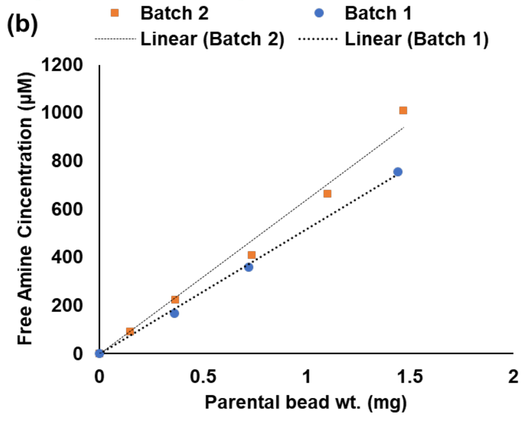
- Acetyl chloride (C2)
- Butyryl chloride (C4)
- Hexanoyl chloride (C6)
- Octanoyl chloride (C8)
- Decanoyl chloride (C10)
- Lauroyl chloride (C12)
- Myristyl chloride(C14)
- Palmitoyl chloride(C16)
- 2-ethyl butyryl chloride (EthylButryl)
- Hydrocinnamoyl chloride (Hydrocinnamoyl)

Parental Amikabead synthesis: Emulsion polymerization between Amikacin hydrate and PEGDE. Characterization with Ninhydrin assay.
Lipid modification: Different chain lengths of lipids (C2, C4, C6, C8, C12) have been conjugated on the parental Amikabead by using aqueous phase Comu-catalyzed[2] reaction.

Experimental Findings

Characterization of Parental Amikabead by Ninhydrin Assay:

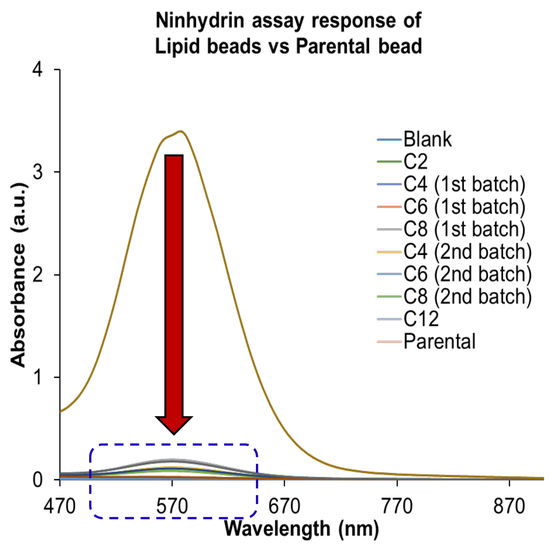


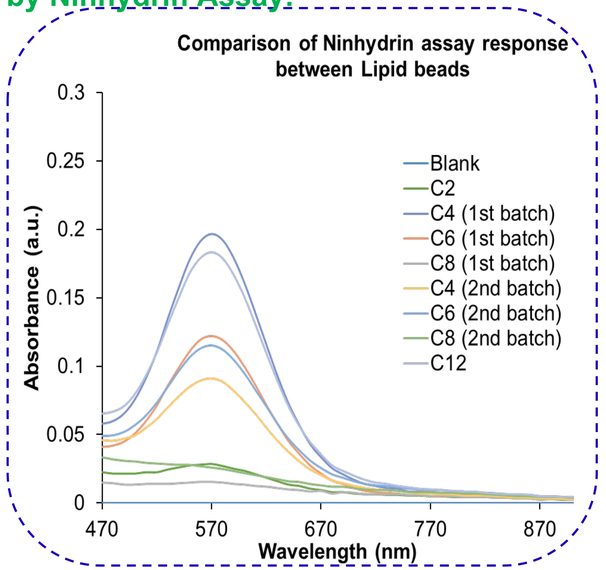


Schematics

Experimental Findings

Characterization of Lipid-modified beads by Ninhydrin Assay:





Obstacles

A significant obstacle that was encountered during the semester was time. There was a significant amount of research to be done and different types of experiments to learn and then execute. Because of this it was difficult to be able to make mass progress in two months.

Conclusion

- Parental Amikabeads were synthesized and characterized successfully with Ninhydrin assay.
- Different chain lengths of lipids were successfully conjugated on the Parental Amikabeads.
- Lipid beads showed a significant drop in Ninhydrin assay response confirming a successful conjugation.

Acknowledgements

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References

1. Aminoglycoside Antibiotic-Derived Anion-Exchange Microbeads for Plasmid DNA Binding and in Situ DNA Capture. *ACS Applied Materials & Interfaces* **2014**, *6 (21)*, 18577-18589.
2. Amide and Peptide Bond Formation in Water at Room Temperature *Organic Letters* **2015**, *17 (16)*, 3968-3971.

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