Synthesis of functionalized peptoids designed to coordinate to quantum dots for use in biomedical applications

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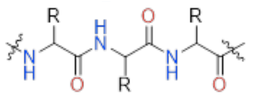
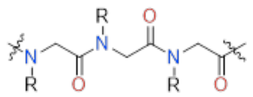
Abstract

Quantum dots are a potential replacement for traditional organic fluorophores. The dots' inherent toxicity is the main barrier limiting biomedical applications, but designed peptoids could provide a solution for this. The objective of this research was to improve the biocompatibility of quantum dots through designed peptoids that will coordinate to the quantum dots and polymerize a shell around the dots. Using the Bradley Protocol and the submonomer method, various peptoids were synthesized. One peptoid variant was used to determine the ideal reductive amination conditions. Other variants were used to test coordination to quantum dots and the cytotoxicity of the peptoids against HepG2 hepatocellular carcinoma cells. Data has indicated that both a short-strand peptoid and a longer-length peptoid have been able to effectively coordinate to quantum dots. Testing the cytotoxicity of the three longer-length peptoids against HepG2 cells indicated that the peptoids have low toxicity levels.

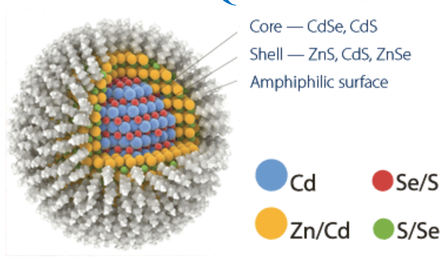
What are Peptoids?

Peptides 

Peptoids 

Peptides are naturally occurring chains of amino acids found in the body. They serve many functions in the body, but their anti-microbial properties are the basis for this work. The issue with peptides is that molecules in the body called proteases recognize and degrade peptides. Fortunately, peptoids have a fundamental shift in the placement of the functional "R" groups that prevents them from being recognized and broken down in the body. This change in structure makes peptoids more useful for *in vivo* applications.

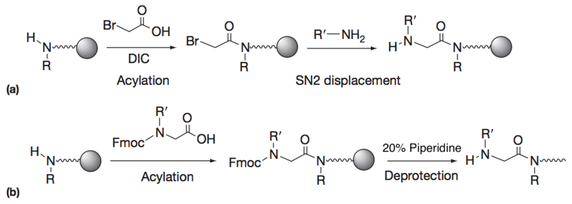
What are Quantum Dots?



Johnston. *Physics World* **2012**.

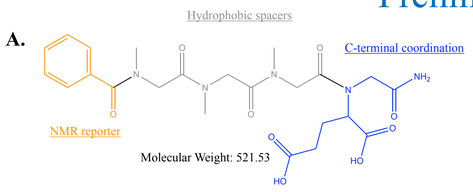
Quantum dots are microscopic nanocrystals, 1 to 20 nanometers wide, with semiconducting properties that allow them to fluoresce when exposed to an excitation light beam. They have strong photostability and show good chemical stability as well, making them a suitable option to replace the traditional organic dyes that are currently used for bioimaging.

Peptoid Synthesis



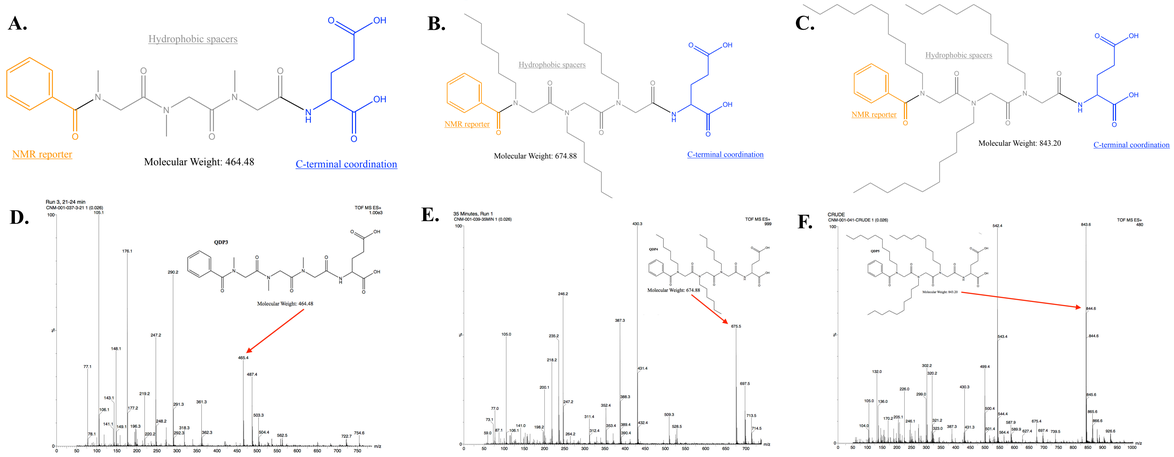
Two common methods of peptoid synthesis. Shown in (a) is the "submonomer method" consisting of a bromoacylation of the terminal amine, followed by a nucleophilic displacement of the bromide by the primary amine being added. Shown in (b) is the "Bradley Protocol" consisting of the amidation of an Fmoc protected amino acid to the solid phase with an activating agent such as HBTU, followed by a deprotection using a 20% Piperidine solution.

Preliminary Peptoids



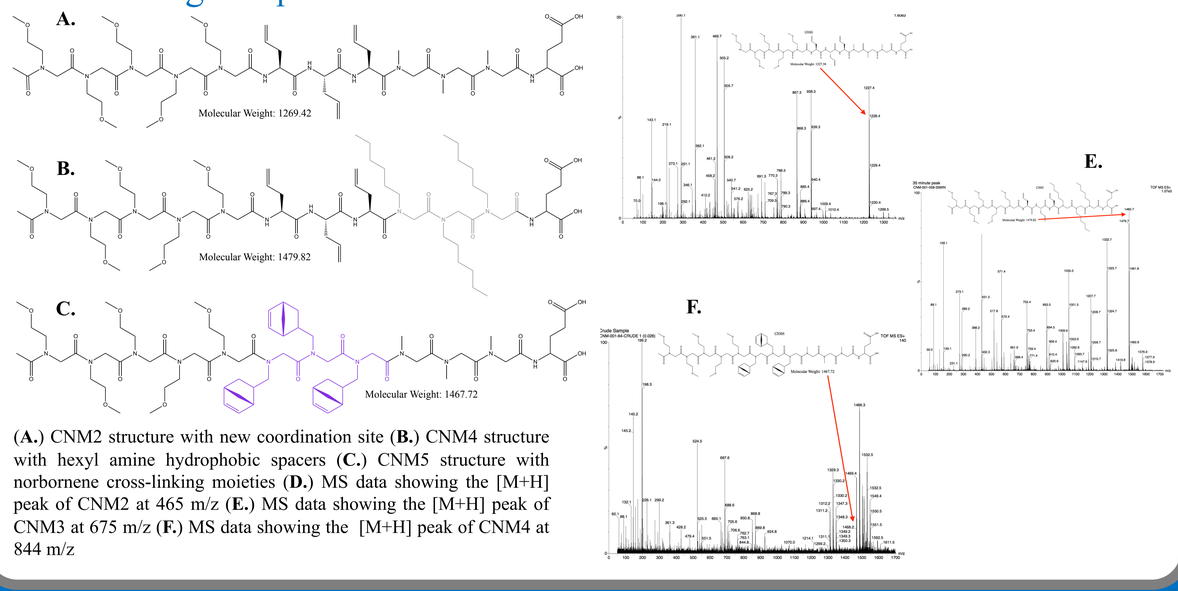
(**A.**) Quantum Dot Peptoid 1 (QDP1) structure with a proposed coordination site, hydrophobic section, and NMR reporter for identification. (**B.**) QDP2 structure used for testing reductive amination conditions (**C.**) Mass spectroscopy (MS) data showing a peak at 522 m/z which corresponds to the [M+H] of QDP1, confirming successful synthesis and purification (**D.**) MS data showing an M+H peak of QDP2 at 605 m/z.

QDP1 Derivatives



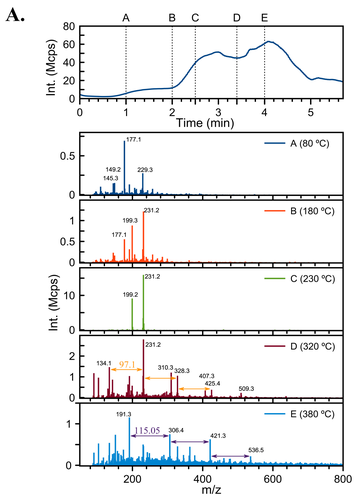
(**A.**) QDP3 structure with new coordination site (**B.**) QDP4 structure with hexyl amine hydrophobic spacers (**C.**) QDP5 structure with decyl amine hydrophobic spacers (**D.**) MS data showing the [M+H] peak of QDP3 at 465 m/z (**E.**) MS data showing the [M+H] peak of QDP4 at 675 m/z (**F.**) MS data showing the [M+H] peak of QDP5 at 844 m/z

Full-Length Peptoid Derivatives



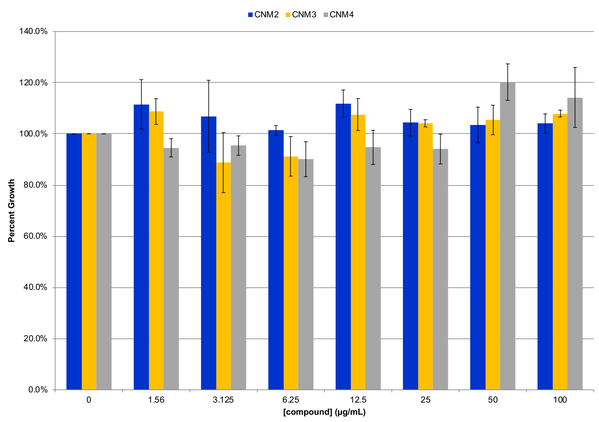
(**A.**) CNM2 structure with new coordination site (**B.**) CNM4 structure with hexyl amine hydrophobic spacers (**C.**) CNM5 structure with norbornene cross-linking moieties (**D.**) MS data showing the [M+H] peak of CNM2 at 465 m/z (**E.**) MS data showing the [M+H] peak of CNM3 at 675 m/z (**F.**) MS data showing the [M+H] peak of CNM4 at 844 m/z

Coordination Testing Using CNM2



(**A.**) DART-MS spectra from CNM2 coordination testing. Repeats of the methoxyethylamine groups (red) of CNM2 are represented by peaks differing by 115.05 mass units in the bottom panel. Repeats of the allyl glycine groups (green) of CNM2 are represented by peaks differing by 97.1 mass units in the second panel from the bottom. (**B.**) Structure of CNM2 with sections of submonomer repeats labeled (methoxyethylamine, red; allyl glycine, green)

HepG2 Cytotoxicity Testing



HepG2 hepatocellular carcinoma cell toxicity test results are shown as percent growth values for each of the three compounds (CNM2, CNM3, CNM4). The three compounds were tested at increasing concentrations from 0 µg/mL up to 100 µg/mL. The percent growth value for a compound at a certain concentrations represents the average of the values from two separate assays. The averages for each compound at each concentration were calculated in this way. The average percent growth for all three compounds indicated that the peptoids were relatively non-toxic at concentrations at or below 100 µg/mL.

Acknowledgments

This research would not have been possible without the guidance from my thesis director, Dr. Kevin L. Bicker of the MTSU Chemistry Department. I also want to thank Dr. Gregory Van Patten who provided the vital quantum dot data from coordination testing he conducted.