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

by
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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.

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Terms and Definitions

1. Peptides — strings of amino acids condensed together that combine and fold to form proteins.
2. Peptoids — N-substituted glycines; mimics of peptide structures that incorporate functional “R” groups on the amide nitrogen as opposed to peptides that have the R groups on the alpha carbon.
3. Quantum Dots — microscopic nanocrystals, 1 to 20 nanometers wide, with semiconducting properties that allow them to fluoresce when exposed to an excitation light beam and that also exhibit strong photostability and chemical stability when compared to organic fluorophores.
4. Biocompatibility — the ability of a foreign material or substance to exist inside of a biological organism and produce a desired effect without producing adverse consequences.
5. Polymerization — a process in which single monomers, or terminal alkenes, are reacted with an initiating species that leads to propagation and the subsequent formation of connected chains
6. Reductive Amination — the reaction of a carbonyl group, such as pentanal, with a primary amine; this forms an intermediate imine that later reduces to form a secondary amine.
7. Submonomer Method — a method of solid-phase peptoid synthesis that consists of a bromoacylation of a terminal amine on a solid material, followed by a nucleophilic displacement of the added bromide by a primary amine.

8. Bradley Protocol — a method of solid-phase peptoid synthesis that consists of the amidation of an Fmoc protected amino acid to a solid phase with an activating agent such as HBTU, followed by a deprotection using a 20% Piperidine solution.

INTRODUCTION

Currently the medical community relies heavily on organic fluorophores to conduct a wide range of bioimaging for treatments and diagnoses.[1] However, these molecules have poor photostability and are not effective for imaging with multiple colors due to their constrained optical properties.[1] 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.[2] They have strong photostability and show good chemical stability as well, making them a suitable option to replace traditional organic dyes.[3]

Quantum dots typically consist of a core semiconducting material that is coated with another semiconducting material that is compositionally graded around the core.[2] These layered materials work together to improve conductivity, to improve chemical and photostability, and to reduce toxicity from the materials that make up the quantum dot cores.[4] Unlike organic dyes which only emit single color spectra when excited by a given wavelength of light, quantum dots have the ability to emit various different light spectra from the same wavelength of light due to their tunable optical properties.[1] Quantum dots' optical properties can be manipulated by changing the material that they are made of, the size of the dot, and the thickness of the shell.[3]

Current bioimaging is done almost exclusively in the visible light range, which is disadvantageous due to light scattering, poor fluorophore emission through tissue due to the absorption of excitation and emission light by surrounding tissue, and autofluorescence of surrounding tissue within the viewing field.[4] Quantum dots offer a possible solution to this issue because of their ability to emit near-infrared light. Using near-infrared (NIR)

quantum dots allows imaging to be collected within ranges of higher wavelengths outside of the visible light range where tissue absorption and autofluorescence starts to decrease drastically.[4]

Bioimaging is a major field of research concerning quantum dots and their future uses in the biomedical field. However, quantum dots have the potential to be useful for a number of other medical applications. One alternative application of quantum dots relates to pharmaceuticals and targeted drug therapy. Some dots are being synthesized with ligands used to deliver medications into targeted regions *in vitro* where the quantum dot fluorescence can be used to track drug delivery.[2] Research is also being done to formulate what are called "site-targeted nanotherapeutics" that seek out diseased areas and deliver genetic, radioactive, or chemotherapeutic treatments only in targeted regions while leaving other surrounding tissue undamaged.[5]

Another growing field involving quantum dots relates to biosensing and the medical advantages of early detection in disease treatment and prevention. Quantum dots can be coupled to specific biomolecules that are then dissolved in water and currently used *in vitro* to detect the presence of targeted biomarkers.[6] Immunosensors are one type of biomarker, referring to antigens given off by pathogens that can be detected by quantum dots functionalized with complementary antibodies coupled to the dots.[3] Genetic sensing is another field growing out of quantum dot research. It centers around the observation and identification of genetic markers connected to disease development through the use of quantum dots functionalized with DNA strands complementary to known genetic mutations.[7]

Whether it is bioimaging, drug delivery, nanotherapeutics, or biosensing, quantum dots are key to the latest research and development in these fields. The main issue with quantum dots, and the limiting factor of quantum dot research in all of the aforementioned fields, is the toxicity and inferred lack of biocompatibility found in numerous types of quantum dots including cadmium-selenide dots, one of the more commonly used dots in research.[8] Without a solution to this issue, future medical research involving quantum dots could mostly remain *in vitro* and may never make it to *in vivo* studies involving humans.

The toxicity of quantum dots stems from the inherently toxic metals that make up the most commonly used dots such as the cadmium-selenide dots or indium-phosphide dots.[8] Currently dots are often coated with zinc sulfide, silica, polymers, or other non-toxic layers to help encase the more toxic cores and to aid in functionalization.[1] However, there is an ongoing search for alternative methods in which quantum dots can simultaneously be functionalized while their toxicity is reduced.

One study published in 2006 specifically researched the effects of coating quantum dots with peptides so that the dots could be used for biological applications.[4] Peptides are strings of amino acids condensed together that combine and fold to form proteins.[9] They can be used to functionalize quantum dots as the aforementioned research has shown, but they are not without flaw. There are active proteases found throughout the body's circulatory system that are tasked with activating biological molecules such as enzymes and hormones through the selective cleavage of amide bonds within inactive proteins.[10] Those same proteases would break down synthesized peptides put into the body, such as ones used to encase and functionalize quantum dots. One potential alternative to peptides are the peptidomimetics known as peptoids, or N-substituted glycines. Peptoids mimic

peptide structures but incorporate functional "R" groups on the amide nitrogen as opposed to peptides that have the R groups on the alpha carbon (**Figure 1**). This fundamental shift in composition allows peptoids to avoid the proteolytic degradation that breaks down peptides, substantially increasing the half-life of peptoids *in vivo*.[11] This means that peptoids could be a possible solution to the shortcomings of peptides in their use of functionalizing and detoxifying quantum dots for biomedical use. In order for quantum dots to be useful in *in vivo* medical applications, they must be soluble in water, non-toxic to the body, and must be able to be functionalized in some manner.[4] Peptoids have the potential to meet all of these requirements. They could be designed to be water soluble, to include a portion of the coupled peptoid to polymerize and encase the toxic materials of quantum dots within a "shell," and to include terminal functional groups for specific biomedical uses. Because of peptoids' durability and versatility, it is our hope that a designed peptoid will help to improve the biocompatibility of quantum dots so that the dots may be used for future medical applications.

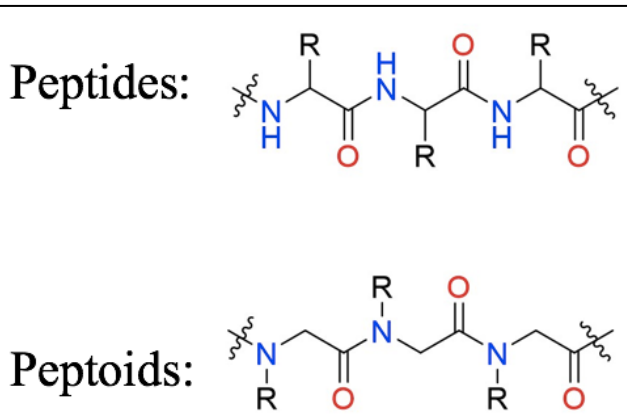

Figure 1: In peptides, the functional "R" group is found on the alpha carbon to the right of the amide nitrogen. Peptoids have the functional R group bonded to the amide nitrogen opposed to the alpha carbon.

RESEARCH OBJECTIVE

The main objective of this research is to improve the biocompatibility of quantum dots through the synthesis of a designed peptoid that will coordinate to the quantum dots, polymerize to form a shell around the dots, and aid in water solubility of the dots. This goal is being pursued to advance the potential medical applications of quantum dots, such as bioimaging and delivery of therapeutics. The aim of this research is to synthesize various functionalized peptoids that will have low cytotoxicity, will coordinate to quantum dots, and will eventually encase and solubilize the dots for future biomedical applications.

METHODOLOGY AND RESULTS

This research utilized solid phase synthesis in order to build numerous peptoid structures that were used to conduct coordination testing and toxicity assays. In regard to this research, solid phase synthesis refers to the successive addition of selectively protected molecules onto a solid material in order to build a desired chain of submonomers. These successive additions allow for selective variability within each step of the synthesis. This is beneficial in the synthesis of peptoids because it allows researchers to design peptoids to serve specific functions not only as collective structures, but also as localized segments within the peptoids.

Protocol for Peptoid and Peptide Synthesis

Two different methods of solid phase synthesis were utilized in this research, first using Fmoc-protected Rink Amide beads as the solid phase before research transitioned to using Wang resin beads instead. The first method, the submonomer method, is also commonly referred to as basic peptoid synthesis (**Figure 2**).[12] This method begins with the addition of a resin sample into a synthesis tube. The resin is then treated with

dimethylformamide (DMF) for 30 minutes to swell the beads. Following this, the resin is treated two times with 20% piperidine in DMF for 10 minutes in order to remove the terminal Fmoc protecting group present on both the Rink Amide beads and the Wang resin beads. The resin is drained and washed with DMF three times and a Kaiser test is performed to test for deprotection. A Kaiser test is performed by collecting a sample of resin beads into a centrifuge tube, adding 75 μL of ninhydrin, centrifuging the sample briefly, and placing the tube into a laboratory oven for 45 seconds. A positive test confirms deprotection and is indicated by the resin turning a purple shade, while a negative test result is shown by the resin remaining a clear color. If a test comes back negative, then the resin is treated with 20% piperidine in DMF two more times for 10 minutes each. Following a positive Kaiser test, a 2 M solution of bromoacetic acid (BrAcOH) and a 3.2 M solution of diisopropylcarbodiimide (DIC) are each independently prepared in anhydrous DMF. The solutions are combined with the Rink Amide resin tube, shaken, and microwaved at 10% power for 15 seconds two times. The reaction is placed on a rocker for 15 minutes and is subsequently washed with DMF 3 times. This step of the reaction bromoacylates the terminal amine on the resin; the appropriate amine will later replace the terminal bromide in a nucleophilic displacement. A Kaiser test is used to check the coupling of the bromoacetic acid to the terminal amine. A positive test indicates that some of the terminal amines were not coupled, and the bromoacylation steps need to be repeated. Following a negative test, a 2 M solution of the appropriate amine is prepared in anhydrous DMF. The amine solution is added to the resin, shaken, and microwaved at 10% power for 15 seconds two times. The reaction is then allowed to rock for 15 minutes and is washed with DMF three times. The nucleophilic displacement of the bromide by the added amine is tested

using a Kaiser test. If the test comes back positive, then the coupling was effective and peptoid synthesis can be repeated by completing the bromoacylation steps followed by the addition steps for other desired amines.

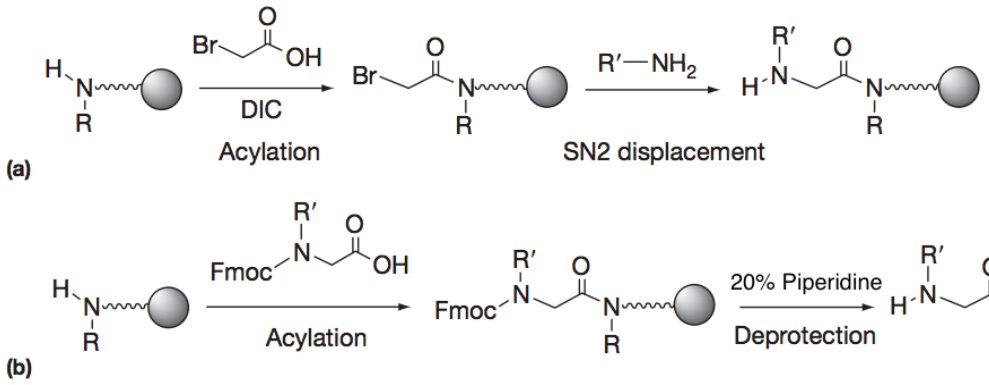

Figure 2: Two common methods of peptoid synthesis. Shown in (a) is the "submonomer method" consisting of a bromoacylation of the terminal amine, followed by a nucleophilic displacement of the bromide by the primary amine being added. Shown in (b) is the "Bradley Protocol" consisting of the amidation 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.

The second method, the Bradley Protocol, is also known as the peptide synthesis protocol (**Figure 2**).[13] This method begins exactly as the submonomer method does with the swelling and deprotection of the resin beads as described above. Instead of performing a bromoacylation and subsequent amine additions, peptide synthesis consists of the activation of Fmoc protected amino acids followed by the addition of an activated amino acid solution to the resin. In a tube separate from the resin, the desired Fmoc protected amino acid and an activating agent such as N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) or (1-cyano-2-ethoxy-2-

oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) are dissolved in 5% N-methylmorpholine in DMF. The solution is shaken until all of the solute is dissolved and is left to sit out and react for 10 minutes. The activated amino acid solution is then added to the resin tube, shaken, and rocked for 1 hour. The resin is drained and washed with DMF 3 times, and the amidation of the amino acid onto the terminal amine of the resin is tested using a Kaiser test. If the test is negative, then further peptide synthesis can be done by first conducting the Fmoc deprotection steps with piperidine followed by the activation and addition steps for other appropriate Fmoc protected amino acids.

Preliminary Peptoid Designs and Testing

The two previously described methods of synthesis were used in combination to synthesize two different preliminary peptoid structures, Quantum Dot Peptoid 1 (QDP1) and Quantum Dot Peptoid 2 (QDP2). Each of the two structures were synthesized to test and optimize different parts of the preliminary methodology so that future work will be more efficient. QDP1 was synthesized in three stages, reflective of its three components: the C-terminal coordination site, the hydrophobic spacers, and the Nuclear Magnetic Resonance (NMR) reporter (**Figure 3**).

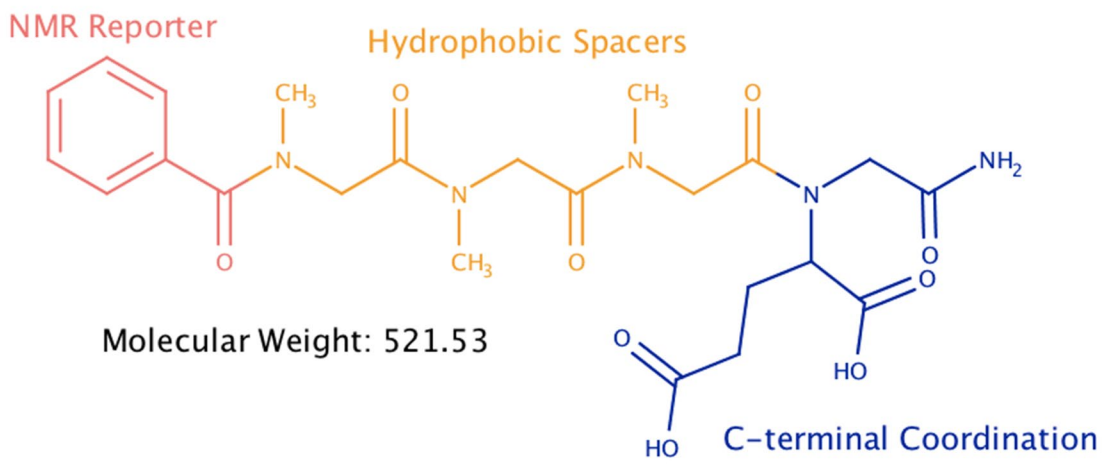

Figure 3: Quantum Dot Peptoid 1 (QDP1), for use in the testing of coordination between peptoid structures and quantum dots.

The synthesis of QDP1 began with the formation of the C-terminal coordination segment using peptoid synthesis. In a 20 mL synthesis column, around 500 mg of Rink amide resin (loading level of 0.75 mmol/g) was combined with DMF and rocked for 20 minutes to swell the beads; the resin was subsequently deprotected using 20% piperidine in DMF. The resin then underwent a bromoacylation using a 5 mL working volume consisting of 2.5 mL of a 2 M BrAcOH solution and 2.5 mL of 3.2 M DIC solution. The two solutions were combined in the resin tube and microwaved twice at 10% power for 15 seconds each. The solution was rocked to react for 15 minutes and subsequently washed with DMF three times. A negative Kaiser test indicated that the bromoacylation was successful. This was followed by a nucleophilic displacement using a 5 mL working volume of NDca solution, which consisted of 1.2 M of L-glutamic acid di-tert-butyl ester hydrochloride (2.96 g) with 3.27 mL of anhydrous DMF and 3.2 M of diisopropylethylamine (DIPEA, 1.73 mL) added in a successive order to a tube separate

from the resin. After the NDca solution was mixed independently, it was added to the resin tube. The resin tube was microwaved for 15 seconds at 10% power two times before it was rocked and reacted for 15 minutes. The tube was drained and washed with DMF three times and a positive Kaiser test indicated successful coupling. This completed the addition of the C-terminal portion onto the Rink Amide resin. This was followed by the formation of the hydrophobic spacer segment using peptide synthesis. In a tube separate from the resin, 1.503 mmol (4 eq) of Fmoc protected sarcosine (Fmoc-Sar-OH) was combined with 1.503 mmol (4 eq) of COMU, a strong activating agent, and was dissolved in 5% N-methlymorpholine in DMF. The solution was added to the resin after 10 minutes of reacting separately. The resin was rocked for 1 hour to allow the solution to react before it was drained and washed with DMF three times. If a positive Kaiser test was observed, then coupling was not successful, and the activation and addition steps were repeated. Once a Kaiser test came back negative, an Fmoc deprotection was conducted through two successive additions of 20% piperidine in DMF to the resin that were each allowed to rock and react for 10 minutes. After each 10-minute reaction, the resin tube was drained and washed with DMF three times. A positive Kaiser test following the second reaction indicated that the Fmoc deprotection was successful and subsequent Fmoc-Sar-OH additions could follow. The activation, addition, and deprotection of Fmoc-Sar-OH was repeated twice more. However, a weaker activating agent called HBTU was used for the second and third sarcosine addition instead of COMU. A stronger activating agent was needed to conduct the first coupling of Fmoc-Sar-OH to the secondary amine of NDca, while HBTU was more cost efficient in subsequent additions when a stronger activating agent was no longer needed. These three sarcosine additions form the hydrophobic spacer

section of QDP1. Following the completion of the hydrophobic spacer component, the final part of QDP1, the NMR reporter segment, was added using peptide synthesis. In a separate tube from the resin, 1.503 mmol (4 eq) of benzoic acid was combined with 1.503 mmol (4 eq) of HBTU and was dissolved in 5% N-methylmorpholine in DMF. After the solution reacted for 10 minutes, the activated solution was added to the resin and rocked for 1 hour. The resin tube was then drained and washed with DMF three times and a negative Kaiser test indicated final coupling was successful. This was the final step in the synthesis of QDP1, so the resin was washed with DCM three times to clear all traces of DMF. To cleave the structure from the resin, a 95% Trifluoroacetic acid (TFA), 2.5% H_2O, and 2.5% triisopropylsilane (TIS) solution was added to the resin tube and rocked for 1 hour. The resulting solution was then filtered from the resin and collected in a new tube. The TFA was evaporated off and the remaining product was resuspended in a 1:1 H_2O to acetonitrile (CH_3CN) solution. High performance liquid chromatography (HPLC) was used to isolate pure samples of the various synthetic products and mass spectrometry identified which sample contained QDP1. The QDP1 sample was identified by an M+1 peak at 522 m/z (**Figure 4)**. The sample of QDP1 was added to a 250 mL round bottom flask and was vacuumed on a rotary evaporator for 20 minutes to evaporate the CH_3CN. The remaining sample was frozen using liquid nitrogen and was placed on a lyophilizer until only a dried, yielding 3.2 mg of QDP1 (yield = 1.6%).

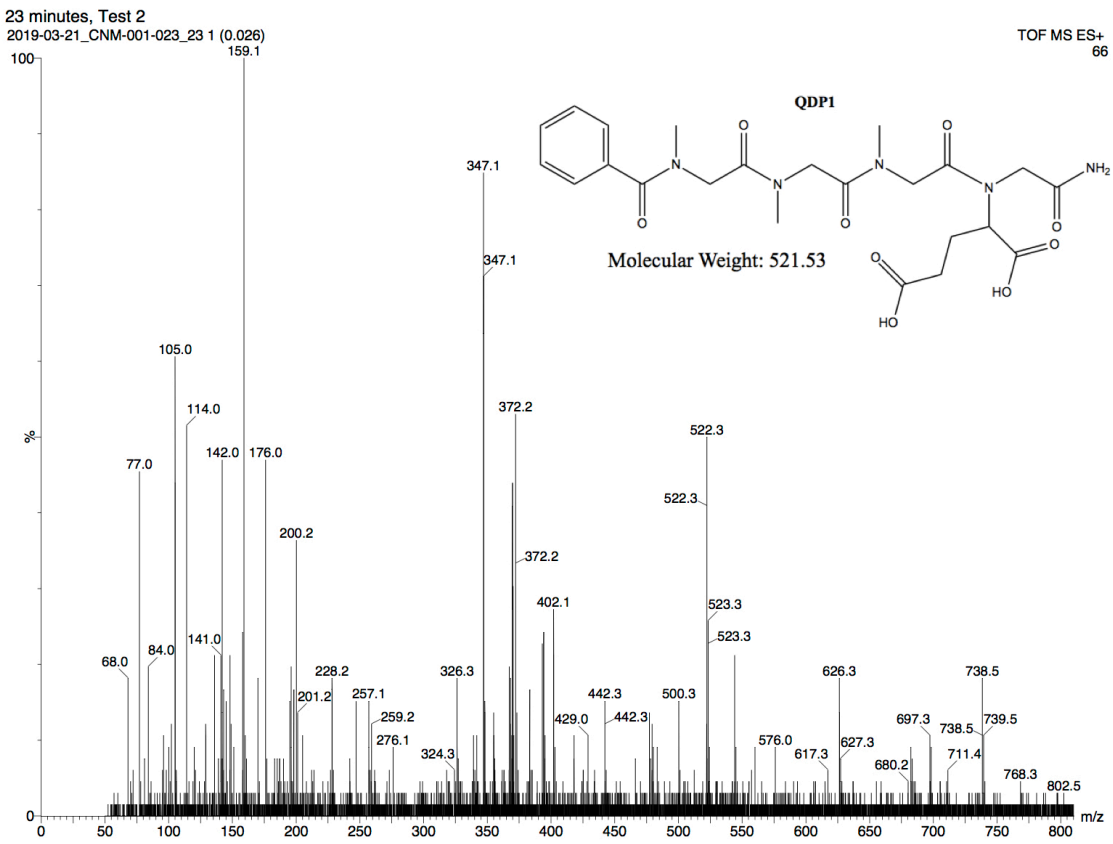

Figure 4: Mass spectrometry testing of QDP1 synthesis, showing a peak at 522 m/z corresponding to the [M+H] of QDP1.

QDP1 was designed and synthesized for later use in testing coordination between the negatively charged C-terminal dicarboxylate and the cations on the surface of quantum dots. However, there was also an interest in determining an efficient procedure to conduct reductive amination, a reaction that could be used to install cross-linking units into the peptoid coated QDs. This was a new research endeavor that was considered to be useful in synthesizing future peptoid designs, so QDP2 was designed to assess which conditions would be best for reductive amination.

The synthesis of QDP2 was conducted in three phases similar to the synthesis of QDP1 (**Figure 5**). The first and largest portion of QD2 was formed using peptoid synthesis.

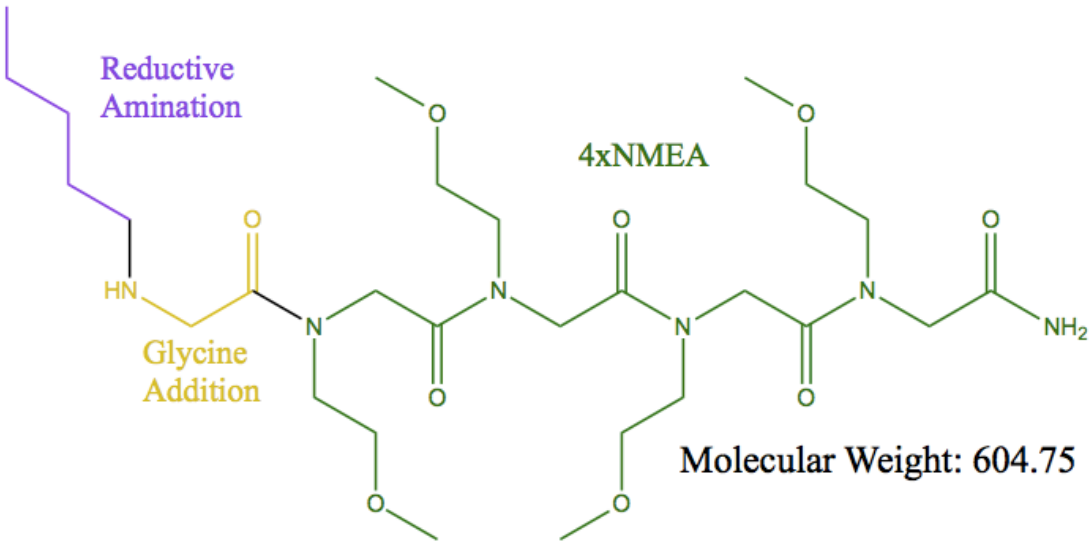

Figure 5: Quantum Dot Peptoid 2 (QDP2), used to optimize reductive amination conditions needed to add the alkene moieties to the complete peptoid.

About 200 mg of Rink amide resin (loading level of 0.75 mmol/g) was treated with DMF for 20 minutes to swell the beads and was subsequently deprotected using 20% piperidine in DMF. The resin then underwent a bromoacylation using 1.5 mL of a 2 M BrAcOH solution and 1.5 mL of a 3.2 M DIC solution. The two solutions were added to the resin tube and the tube was microwaved for 15 seconds at 10% power two times. After this, the resin tube was rocked for 15 minutes and was then drained and washed three times with DMF. A negative Kaiser test indicated a successful bromoacylation. This was followed by the amine addition using a 3 mL volume of a 2 M solution of 2-methoxyethylamine in anhydrous DMF. This solution was also microwaved for 15 seconds at 10% power two

times before it was left to rock and react for 15 minutes. The tube was then drained and washed with DMF three times and a positive Kaiser test indicated that the amine coupling was successful. The bromoacylation and 2-methoxyethylamine addition steps were repeated 3 more times to form the complete 4xNMea portion of QDP2. The next segment of QDP2, the glycine addition, was formed using peptide synthesis. In a tube separate from the resin, 0.609 mmol (4 eq) of Fmoc protected glycine (Fmoc-Gly-OH) was combined with 0.609 mmol (4 eq) of HBTU and was dissolved in 5% N-methlymorpholine in DMF. This solution reacted separately for 10 minutes and then the activated amine solution was added to the resin. After rocking the resin tube for 1 hour, the resin was washed with DMF three times and an Fmoc deprotection was done using 20% piperidine in DMF.

The final phase of QDP2 synthesis was the most important because this was the portion of synthesis that tested reductive amination conditions. The testing was conducted by taking several samples from the resin with the 4xNMea plus glycine already attached (Gly-4xNMea) and subjecting them to different reductive amination methods. The synthesis consisted of two parts, a pentanal addition to the terminal primary amine to form an imine, followed by the reductive amination of the imine to form a secondary amine (**Figure 6**).

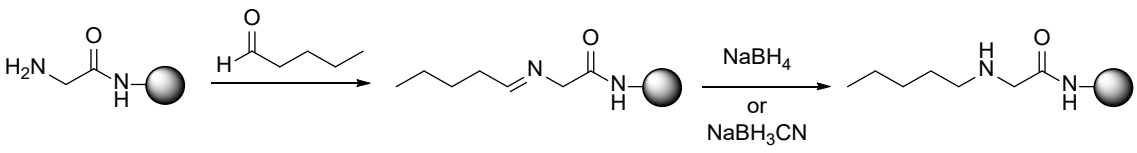

Figure 6. General schematic for reductive amination of an aldehyde onto a solid phase amine. Reaction conditions were varied between different trials.

This would test the feasibility using this chemistry to add 4-pentenal as a cross-linking reagent in a full length QD peptoid. During the first test, these two parts were conducted independently in successive order. A sample of 50 mg of Gly-4xNMea was placed in a new synthesis column. A 9:1 ratio of 1 mL DMF:MeOH was added to the new resin tube. After this, 20 µL (0.114 mmol) of pentanal was added to the resin and the solution was placed on a tumbler inside an incubator at 35°C to run overnight. The following day, 7.8 mg (0.206 mmol) of $NaBH_4$ was added to the resin tube and the solution was shaken and vented every 10 minutes for 30 minutes total to release built up pressure from hydrogen gas. The resin tube was then placed back onto the tumbler inside the 35°C incubator where the reaction was allowed to run for 3.5 hours. The solution was drained, and the remaining resin was washed twice each with increasingly polar solvents from DMF to methanol to water, then back to methanol two times and DMF two times. The resin was then washed with DCM. A portion of the resin was put in a 1.5mL tube and 500 µL of TFA was added to the tube to cleave the product from the resin. After the resin rocked for 30 minutes in the TFA, the TFA was evaporated off and the remaining solution was resuspended in 500 µL of 1:1 H_2O to CH_3CN solution. Mass spectrometry was conducted and a peak at 605 m/z was observed (M+H of QDP2) indicating that the reaction had worked. However, a peak at 535 m/z indicated that some of the resin did not couple with the pentanal, and only included the Gly-4xNea segment of QDP2 (**Figure 7**). Because of this, the decision was made to combine the pentanal addition and the reductive amination steps and to use a more selective reducing agent, $NaBH_3CN$, in the next test.

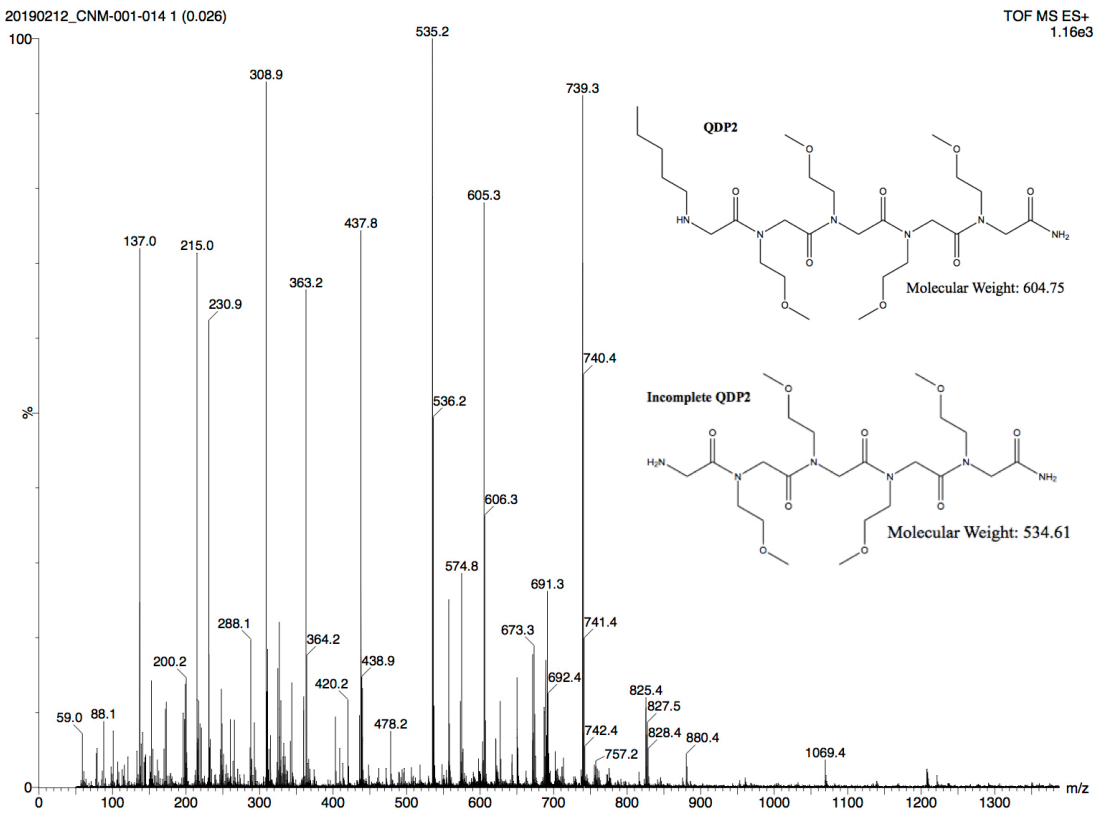

Figure 7. Initial attempt at reductive amination, resulting in partial formation of the desired product (605 m/z) and unreacted starting material (535 m/z)

In a new tube, 50 mg of the Gly-4xNMea resin was combined with a 9:1 ratio of 1 mL DMF:MeOH, 60 µL (0.342 mmol) of pentanal, 10 µL (0.175 mmol) of AcOH, and 35 mg (0.557 mmol) of $NaBH_3CN$. Molecular sieves were also added to the solution to absorb water and push the reaction towards the product. The combined resin solution was shaken and vented every 10 minutes for 30 minutes, then placed on the tumbler inside the 35°C incubator overnight. On the following day, the resin was drained, washed, and cleaved the same way as the first test, and mass spectrometry was run on a sample of the resin. The data came back showing a strong peak at 675 m/z, indicative of a double reductive amination that formed a tertiary amine (**Figure 8**). This meant the reaction worked, but too

effectively as pentanal was adding to the terminal amine to form an imine that then reduced to form a secondary amine. Another pentanal would then add onto the secondary amine again to form another iminium that would reduce to form a tertiary amine.

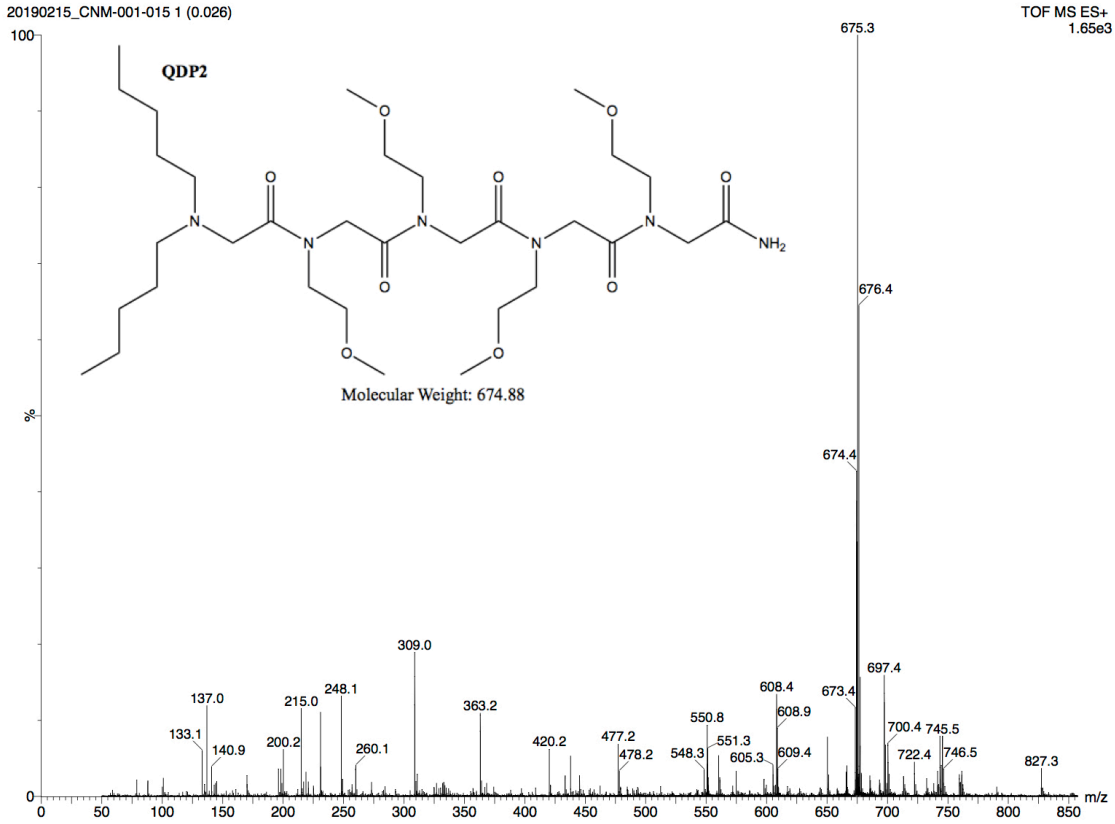

Figure 8. Second attempt at reductive amination, resulting in di-alkylation of the terminal amine (675 m/z).

To counteract this, the reaction was conducted in two parts similar to how the first test was done. For the final test, 50 mg of the Gly-4xNMea resin was combined with a 9:1 ratio of 1 mL DMF:MeOH, 60 µL of pentanal, and 10 µL of AcOH. That reaction ran overnight on the tumbler inside the 35 °C incubator. The following day, the resin was drained to remove unreacted pentanal and a fresh solution of 9:1 ratio of 1 mL DMF:MeOH

was combined in the resin tube with 35 mg of $NaBH_3CN$ and was allowed to react for 3.5 hours. After this, the resin was drained, washed, and cleaved the same way as the other two previous tests, and mass spectrometry was run on a sample of the resin. Mass spectrometry results confirmed the presence of the completed QDP2 in the resin solution with a peak at 605 m/z (**Figure 9)**. The research involving QDP2 provided insight into some of the various ways that reductive amination can be conducted. After the optimal conditions for this process were determined, research involving QDP2 was considered complete and no further testing on reductive amination was done.

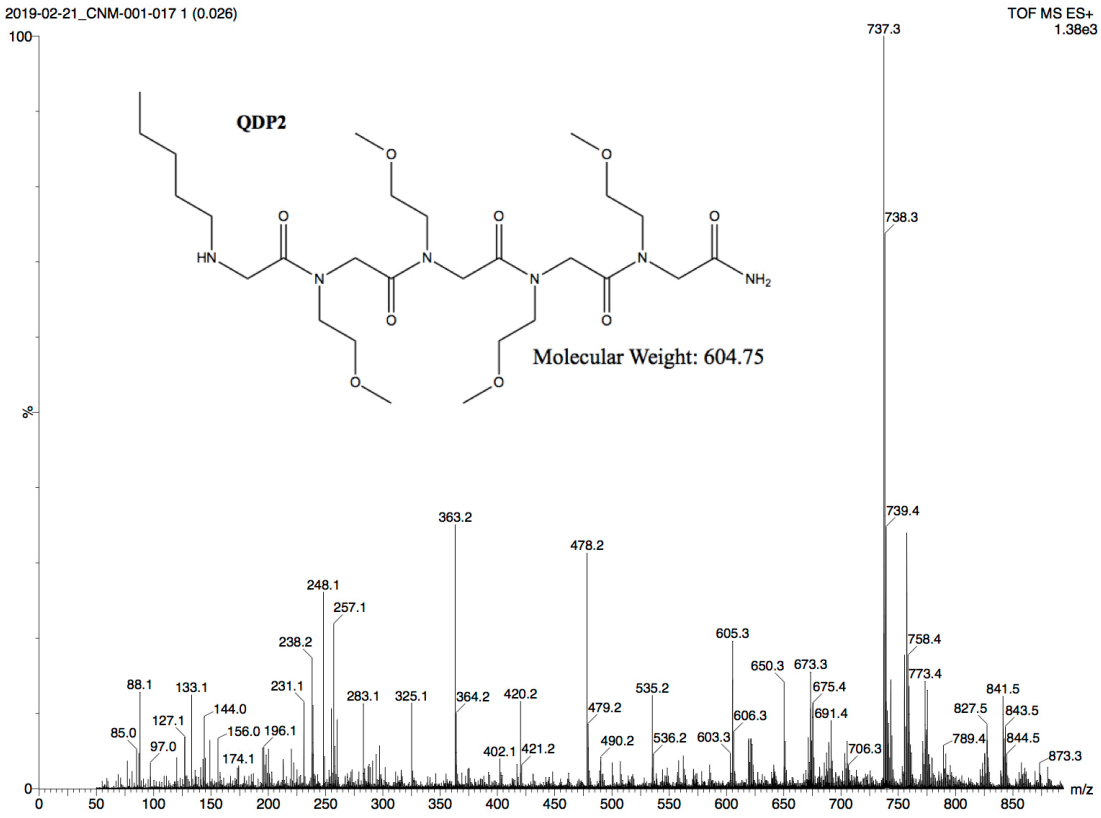

Figure 9: Mass spectrometry testing of QDP2 synthesis, showing a peak at 605 m/z corresponding to the [M+H] of QDP2.

Synthesis and Coordination Testing of QDP1 Derivatives

Since reductive amination testing with QDP2 was complete, research stemming from QDP1 became the main focus moving forward. The synthesis and purification of QDP1 yielded a purified sample of peptoids that was used for preliminary coordination testing conducted by Dr. Gregory Van Patten. The testing confirmed that the C-terminal dicarboxylate coordination site on QDP1 molecules could effectively coordinate, or bind, to the surface of the quantum dots. However, the process of synthesizing the C-terminal coordination site was tedious and inefficient. This difficulty, combined with an interest in the effects of varying hydrophobicity, led to the development of three derivatives of QDP1 that were synthesized for further testing. The synthesis of the three QDP1 derivatives—Quantum Dot Peptoid 3 (QDP3), Quantum Dot Peptoid 4 (QDP4), and Quantum Dot Peptoid 5 (QDP5)—all utilized a Wang resin that had a slightly altered C-terminal coordination site already bound to the beads. This change in the peptoid structures was done in an effort to improve the yield from synthesis, but the second variation in the new derivatives centered on the hydrophobic spacer section. To test the effects of increased hydrophobicity and size in this region on coordination efficacy, different submonomers were used to form the hydrophobic spacers in QDP4 and QDP5.

The first derivative, QDP3, was a close replicate of QDP1 (**Figure 10**). Since using NDca to form the coordination site on QDP1 led to complications and poor yields, the new Wang resin with an Fmoc-protected glutamate already bound to the bead was used to conduct all future synthesis.

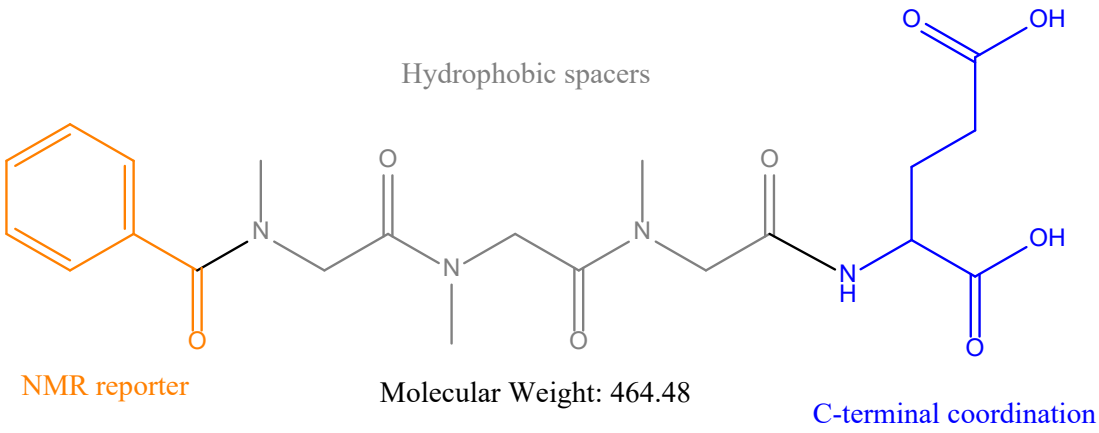

Figure 10. Structure of QDP3, testing an alternative C-terminal coordination moiety.

The advantage of using a Wang resin over a Rink Amide linker is that the final C-terminus after cleavage is a carboxylic acid instead of an amide. By starting with glutamic acid loaded Wang resin, cleavage would result in a di-carboxylate on the C-terminus, giving the desired bidentate ligand to coordinate QDs. The structure of QDP3 included the new C-terminal coordination site, followed by three Fmoc-protected sarcosine (Fmoc-Sar-OH) groups and a benzoic acid group at the N-terminus. Since the new Wang resin already had the Fmoc-protected C-terminal coordination site bound to the beads, actual synthesis began with the addition of the sarcosine groups. First, around 250 mg of Wang resin (loading level of 0.44 mmol/g) was combined with DMF in a new synthesis column for 20 minutes to swell the beads. The resin was subsequently deprotected using 20% piperidine in DMF. After a positive Kaiser test result, the addition of the hydrophobic sarcosine spacers was conducted utilizing peptide synthesis. In a tube separate from the resin, 0.44 mmol (4 eq) of Fmoc-protected sarcosine (Fmoc-Sar-OH) was combined with 0.44 mmol (4 eq) of HBTU and was dissolved in 5% N-methlymorpholine in DMF. The solution was allowed to react separately for 10 minutes before it was added to the resin. The resin tube

was then rocked for 1 hour before it was drained and washed with DMF three times. If a negative Kaiser test was observed after the addition, then an Fmoc deprotection using 20% piperidine in DMF was conducted. This activation, addition, and deprotection of Fmoc-Sar-OH was repeated two more times to create the three hydrophobic spacers of QDP3. After the hydrophobic sarcosine groups were added, the NMR reporter segment was added, again using peptide synthesis. In a separate tube from the resin, 0.44 mmol (4 eq) of benzoic acid was combined with 0.44 mmol (4 eq) of HBTU and was dissolved in 5% N-methylmorpholine in DMF. The solution was left to react separately for 10 minutes before the activated solution was added to the resin and rocked for 1 hour. The resin tube was drained and washed three times with DMF. A negative Kaiser test following this addition indicated that the benzoic acid group had been successfully coupled. QDP3 was then cleaved and purified as previously described and mass spectrometry was used to confirm the QDP3 structure with an M+1 peak at 465 m/z (**Figure 11**). The isolated QDP3 sample was added to a 250 mL round bottom flask and was vacuumed on a rotary evaporator for 20 minutes to evaporate the CH_3CN. The remaining sample was frozen with liquid nitrogen and placed on a lyophilizer until only a dried, pure sample of QDP3 remained. Synthesis of QDP3 yielded 13.2 mg (25.9%). This derivative of QDP1 was used to first test whether the new C-terminal coordination site would still bind to quantum dots. Coordination testing done by Dr. Gregory Van Patten indicated that QDP3 did effectively coordinate to the quantum dots despite the change in the C-terminal site.

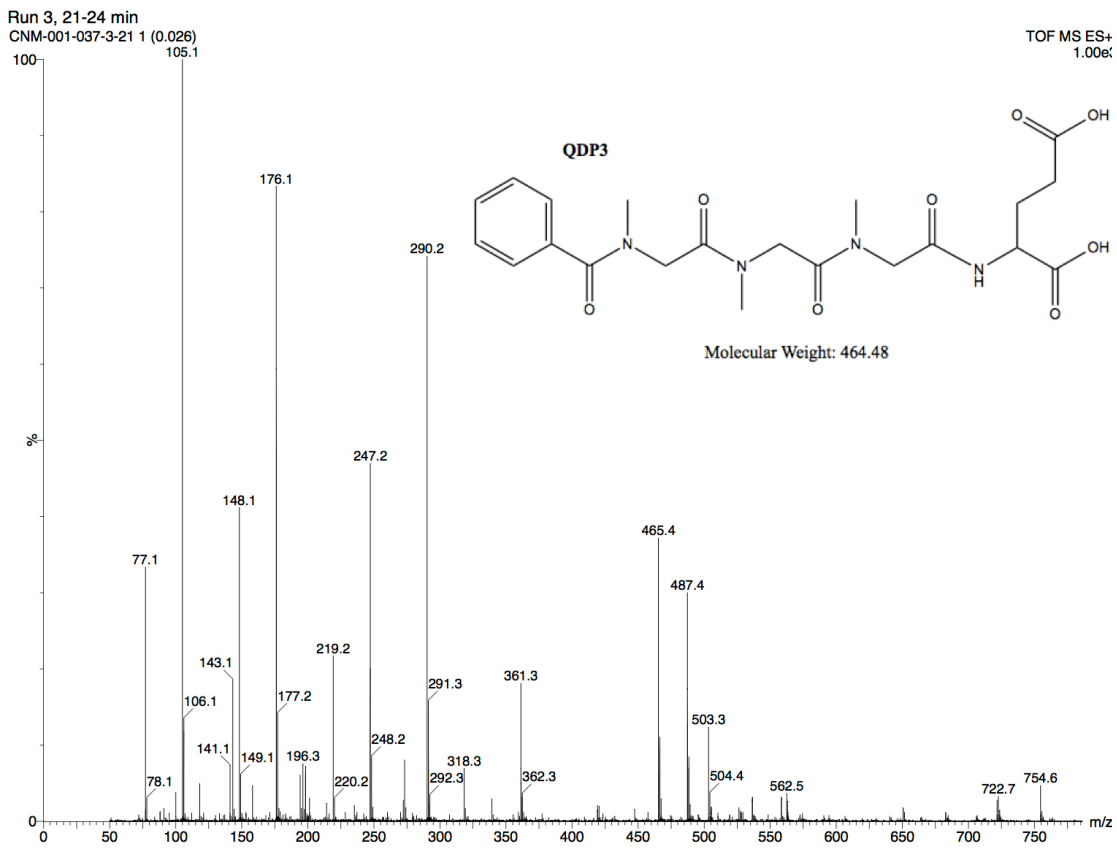

Figure 11. MS spectra confirming the synthesis and purification of QDP3 (465 m/z).

The second derivative, QDP4, had the same C-terminal coordination site and N-terminal benzoic acid group as QDP3. However, QDP4 and QDP5 were designed to include different hydrophobic spacer groups so that the level of hydrophobicity would vary across the three derivatives of QDP1 (**Figure 12**).

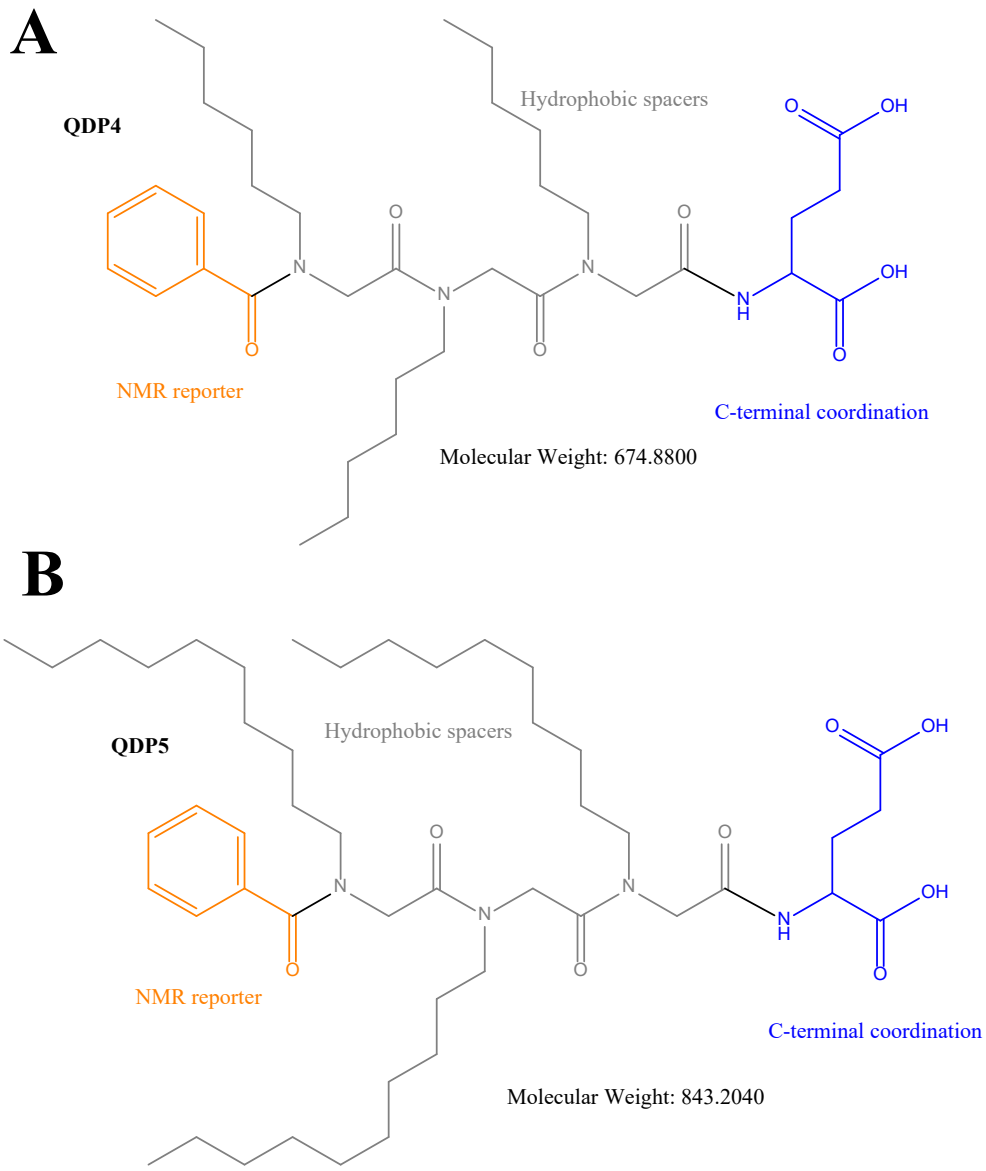

Figure 12. Structures of QDP4 (A) and QDP5 (B) used to test the effect of increased bulk in the hydrophobic spacer section on QD coordination.

QDP4 and QDP5 were synthesized as previously described using the peptoid submonomer synthesis method to install hexyl amine or decyl amine, respectively into the hydrophobic section. These larger alkyl groups increased peptoid hydrophobicity relative to QDP3. HPLC purification and lyophilization yielded pure QDP4 (27.9 mg; 37.6% yield)

and QDP5 (4.1 mg; 4.4% yield). Mass spectrometry was used to confirm the structure of QDP4 ((675 m/z; **Figure 13**) and QDP5 (844 m/z; **Figure 14).** The coordination testing for QDP4 has not yet been completed, but the sample is being saved for future testing conducted by Dr. Gregory Van Patten. The yield for QDP5 was too small to isolate a quantity that was large enough to conduct coordination testing, so this molecule will need to be resynthesized to obtain a sample for future testing.

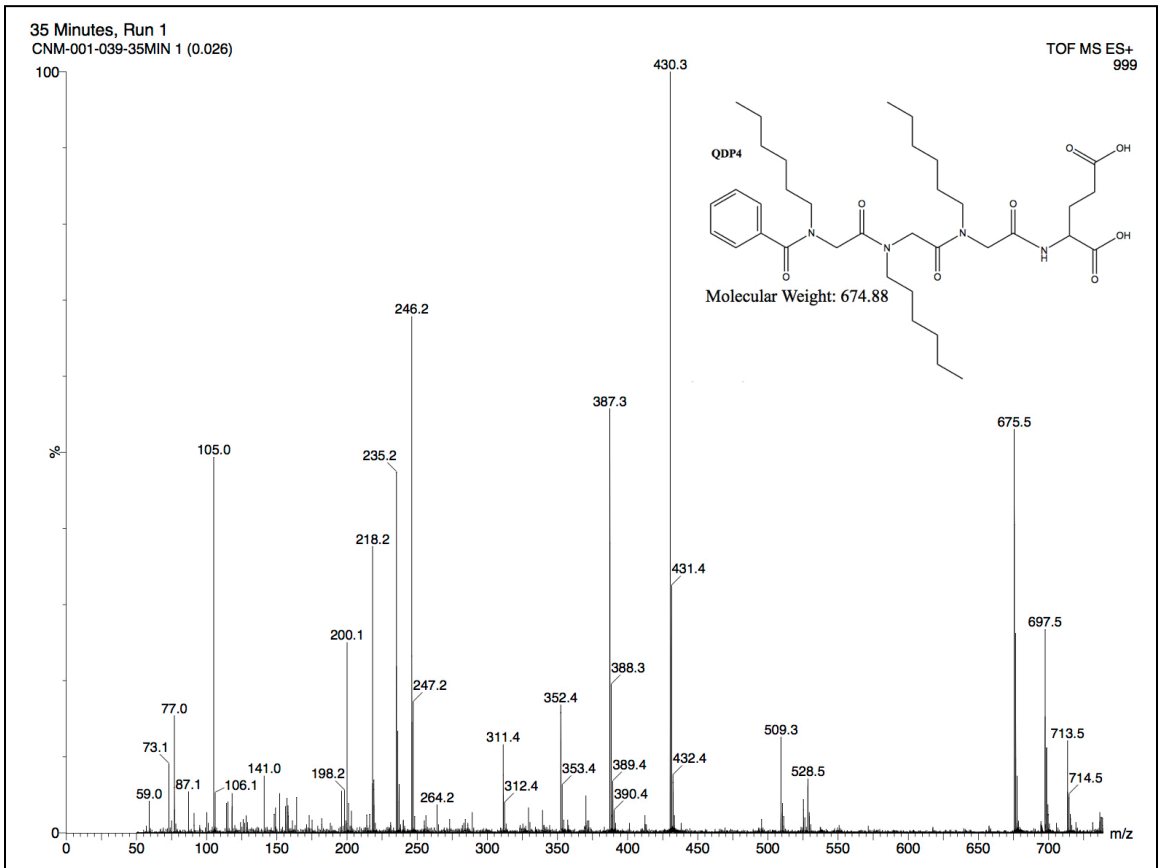

Figure 13. MS spectra confirming the synthesis and purification of QDP4 (675 m/z).

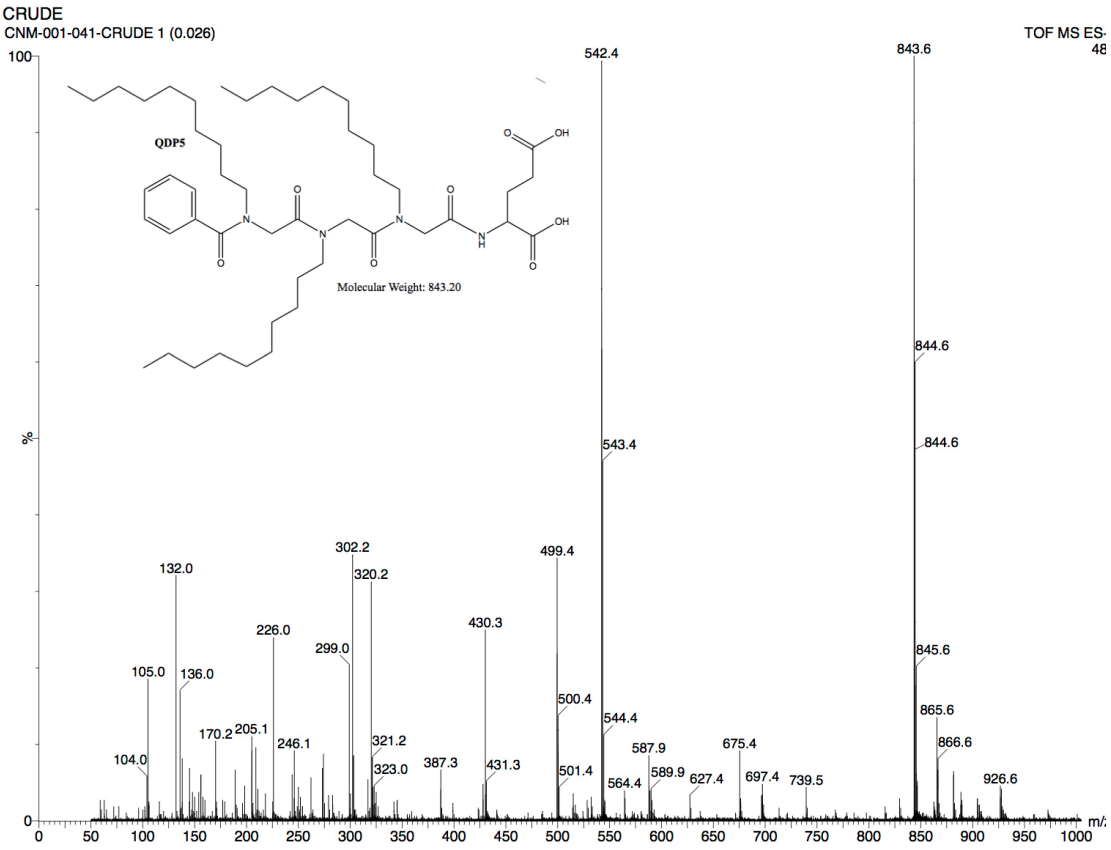

Figure 14. MS spectra confirming the synthesis and purification of QDP5 (844 m/z).

Synthesis of Full-Length Peptoid Variants of the QDP1 Derivatives

Once it was confirmed that varying hydrophobicity levels still showed effective peptoid coordination to quantum dots, it was decided that full length peptoids should be synthesized to push testing further. Longer, more specialized peptoids can include a greater number of functional groups that allow the peptoids to do more than coordinate to quantum dots. Coordination testing using the shorter QDP1, QDP3, QDP4, and QDP5 derivatives showed that the bidentate functional group included in these structures effectively coordinated the peptoids to quantum dots and formed resilient bonds that did not readily deteriorate. The C-terminus from the Wang resin was established as a synthetically easier

way to form a C-terminal dicarboxylate and used for future designed peptoids. A hydrophobic spacer section was also considered to be an important functional group of the peptoids for this research. This functional group forms an anhydrous space around the binding site that prevents degradation of the peptoid-quantum dot bonds when transferred into aqueous media.

The full-length peptoid variants of the QDP3-5 derivatives incorporated two new specialized sections aimed at making the peptoid-quantum dot structures more biocompatible and more water soluble. A cross-linking section consisting of several terminal alkenes off long carbon chains was added to the new variants so that neighboring structures could be polymerized post-coordination to quantum dots. As neighboring peptoids on the surface of the quantum dot polymerize, a plastic "shell" will ideally form to encapsulate the inherently toxic quantum dots. If a complete shell can be developed around the quantum dots, this is expected to increase the biocompatibility of the dots by keeping metal in and water out. The second new section in the full-length variants consisted of five 2-methoxyethylamine submonomers that form a hydrophilic tail. This section was incorporated in an effort to improve the water solubility of the peptoid-quantum dot structures. Improving solubility is important to ensure that the coordinated structures do not crash out of solution and form aggregates that would be toxic in the human body. The first-generation full-length variant was labeled CNM1, and it incorporated both of the new sections as well as the original two sections of the QDP3 derivative (**Figure 15**). While CNM1 was the first proposed full-length peptoid, it was never synthesized or used for testing due to the challenge of synthesizing the highly functionalized cross-linking section. Instead, CNM1 served as a base model for a sequence of derivatives that used varying

submonomers to form the four essential sections. Three derivatives of the CNM1 model—CNM2, CNM3, and CNM4—were synthesized to assess the coordination efficacy and the toxicity of full-length peptoids that could eventually be used in polymerization and biostability testing.

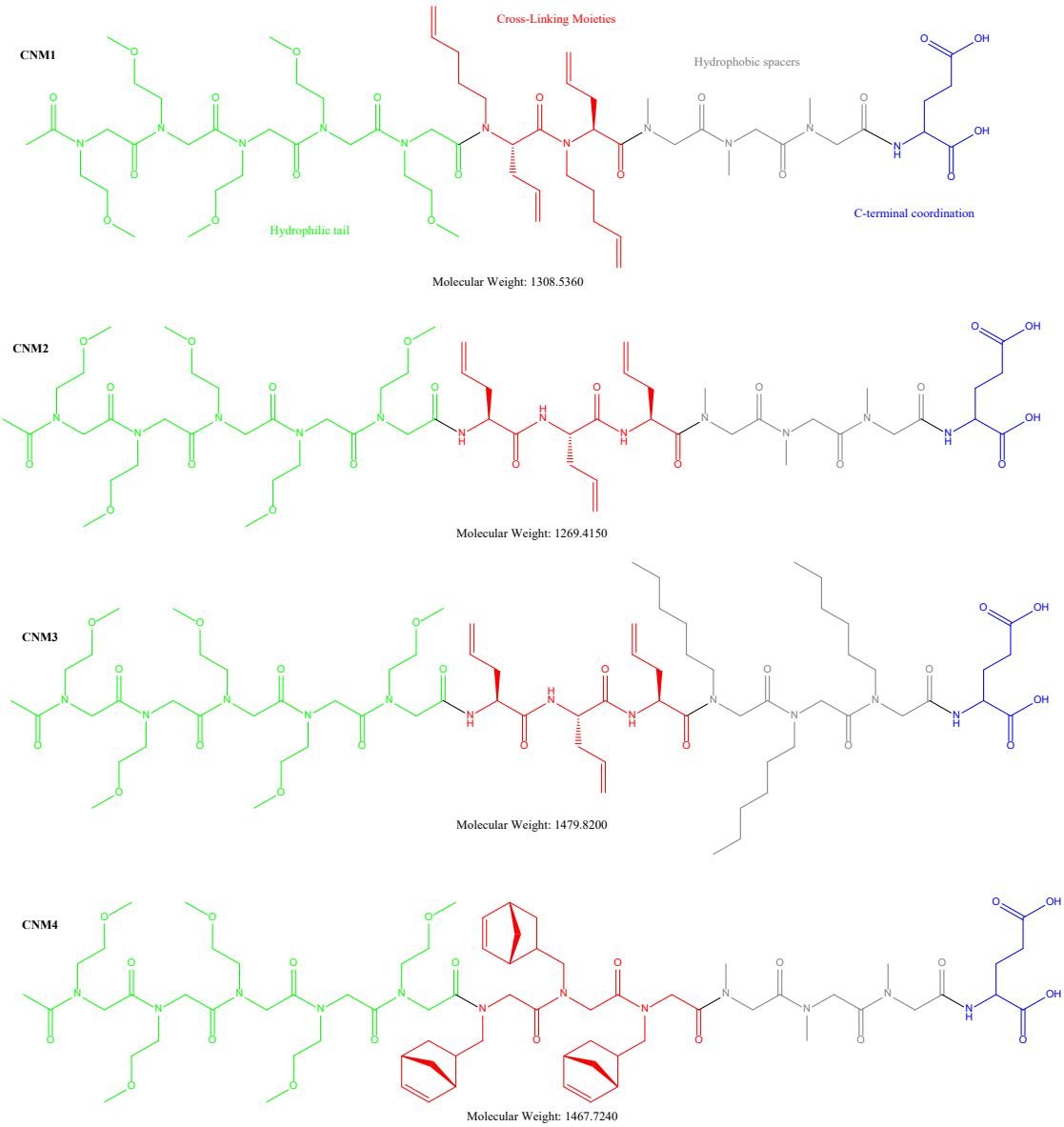

Figure 15. Structures of full length QD peptoids CNM1, CNM2, CNM3, and CNM4, including C-terminal dicarboxylate to coordinate to quantum dots, hydrophobic spacers, cross-linking moieties for polymerization, and a hydrophilic tail.

The first variant, CNM2, resembled the structure of QDP3. The structure of CNM2 started with the negatively charged dicarboxylate that formed the C-terminal coordination site followed by three sarcosine additions to form the hydrophobic spacer section. After the hydrophobic section, three allyl glycine groups were added using Fmoc-allyl-Gly-OH

and the Bradley Method to form the cross-linking moieties component of CNM2. The terminal alkenes on the three submonomers included in this group will be the target for polymerization testing in future research. The structure of CNM2 continues with five additions of 2-methoxyethylamine using the peptoid submonomer method that make up the hydrophilic tail portion of the peptoid. The structure was also intended to end with an acetyl group attached at the N-terminus of CNM2. This was intended to form a "cap" that would prevent N-terminus coordination between CNM2 molecules and the quantum dots. However, the acylation step of the CNM2 synthesis was unintentionally overlooked and the synthesized structure terminated at the fifth 2-methoxyethylamine addition. CNM2 was synthesized and purified using previously described methods to yield 102 mg (37.8%).The CNM2 structure was confirmed using mass spectrometry (1228 m/z) (**Figure 16**). This confirmed that the peptoid had been successfully synthesized except for the acetyl cap that was intended to be on the N-terminus.

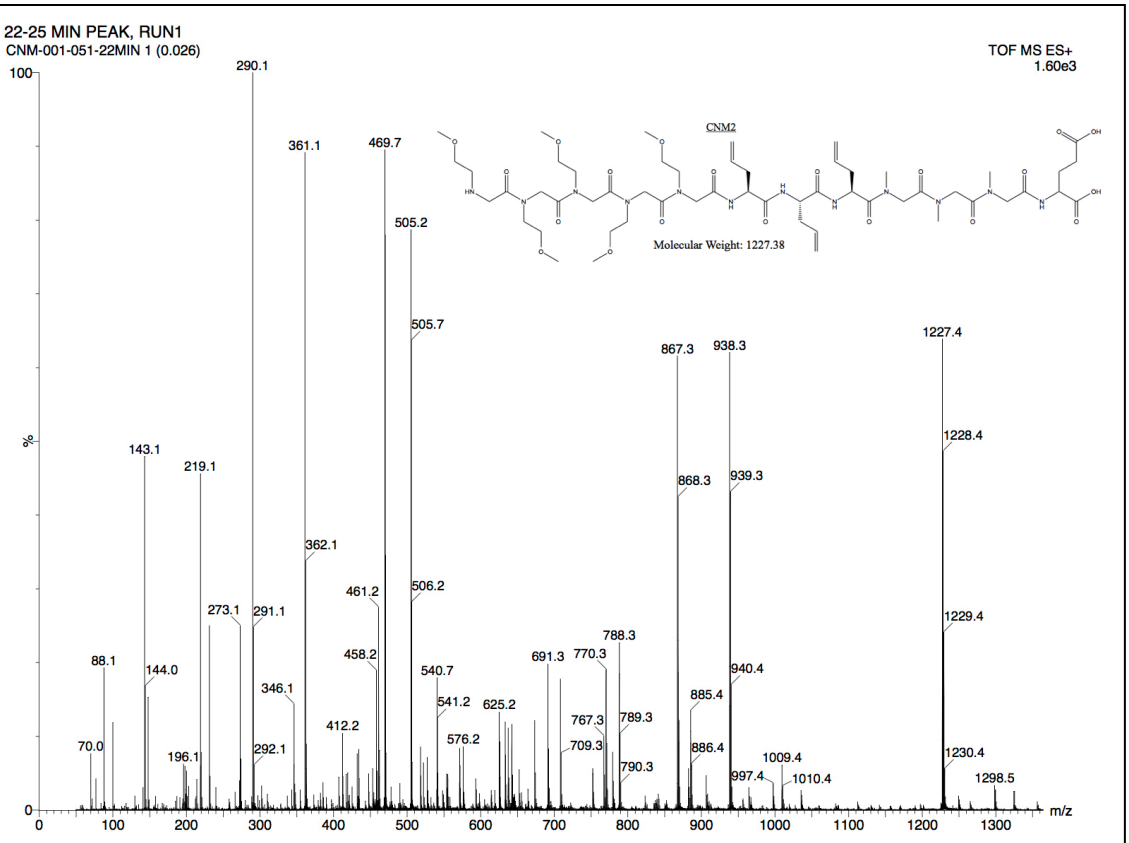

Figure 16. MS spectra confirming the synthesis and purification of CNM2 (1228 m/z).

A sample of the CNM2 derivative was used by Dr. Gregory Van Patten to conduct coordination testing using an aluminum substrate that has a surface chemistry that resembles quantum dots. A solution containing the aluminum substrate and the CNM2 sample was reacted and prepared so that DART-MS could be run. Among the various spectra from the DART-MS, two panels representing spectra collected under high temperature conditions showed promising data (**Figure 17**). The bottom two panels exhibited several peaks that differed by certain mass units that correspond to submonomer groups in CNM2. The bottom panel contained three peaks that each had a difference of 115 mass units, which corresponds to the mass of the methoxyethylamine submonomers of

CNM2. Directly above this panel, there were three peaks that were each 97 mass units apart, which corresponds to the allyl glycine submonomers of CNM2. Both of these peaks indicate the presence of CNM2 in the solution used for DART-MS. If it is assumed that all unbound peptoid was removed after reacting with the aluminum substrate, then the presence of CNM2 within the DART-MS would indicate that the sample coordinated to the substrate.

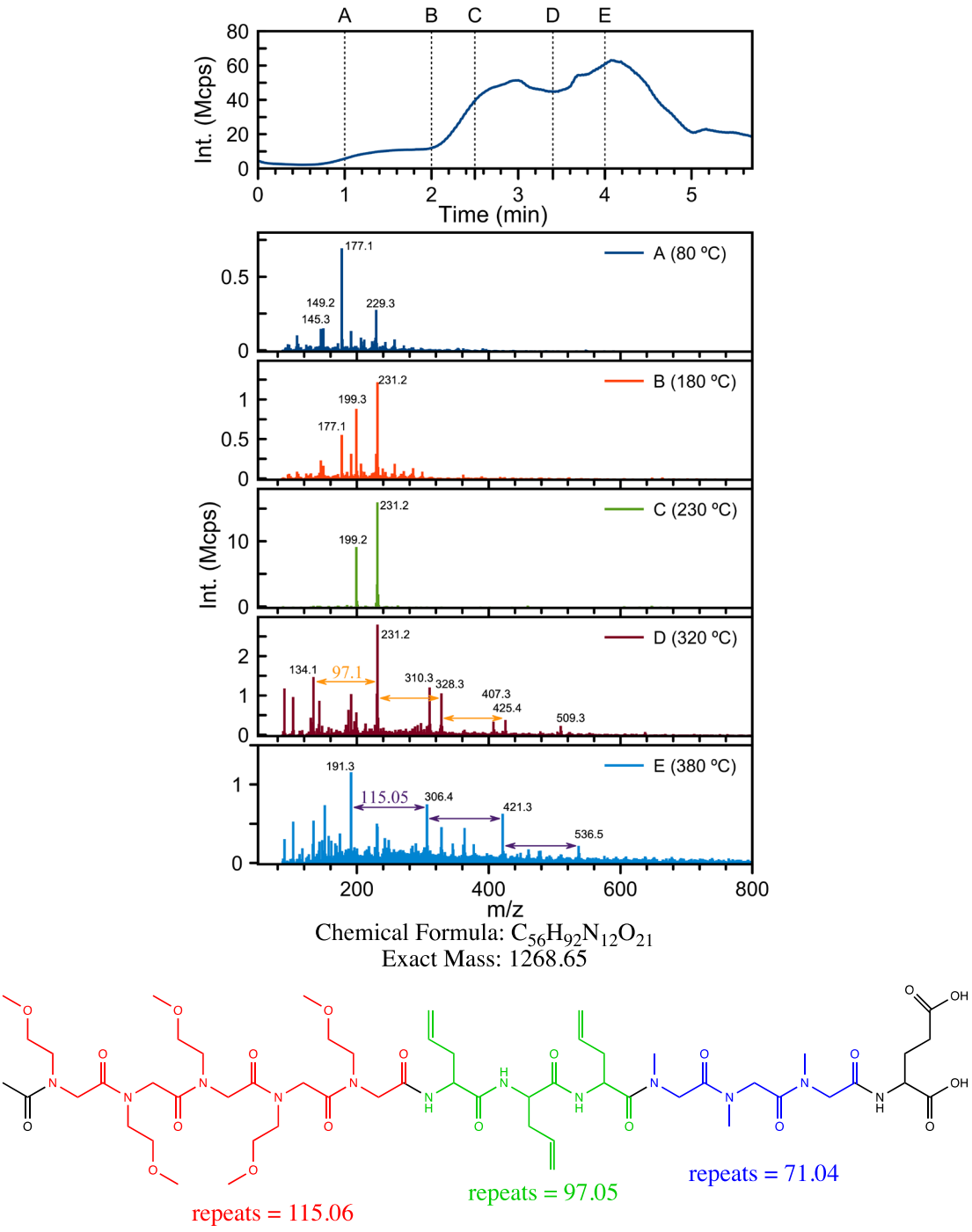

Figure 17. DART-MS spectra from CNM2 coordination testing. Repeats of the methoxyethylamine groups (red) of CNM2 are represented by peaks differing by 115.05 mass units in the bottom panel. Repeats of the allyl glycine groups (green) of CNM2 are represented by peaks differing by 97.1 mass units in the second panel from the bottom.

The second derivative, CNM3, was a replicate of CNM2, except it utilized three hexyl amine submonomers to form the hydrophobic spacer section instead of the Fmoc-protected sarcosine groups (**Figure 15**). The synthesis of CNM3 was completed using previously described methods. The structure of CNM3 was designed to include an acetyl cap on the N-terminus of the peptoid just like CNM2. But unlike the CNM2 synthesis, the CNM3 sample actually included the acetyl cap. Following the last 2-methoxyethylamine addition, a 10 mL solution with 5% acetic anhydride in DCM with 5% pyridine (9mL DCM, 0.5 mL acetic anhydride, 0.5 mL pyridine) was made and was added to the CNM3 resin tube. It was rocked and allowed to react for 1 hour before it was drained and washed with DCM. A negative Kaiser test indicated successful acetylation. CNM3 was then cleaved and purified and described previously to yield 21 mg (6.4%). An M+1 peak at 1480 m/z was observed in the mass spectrometry results for the CNM3 sample, indicating that the complete structure was synthesized (**Figure 18**).

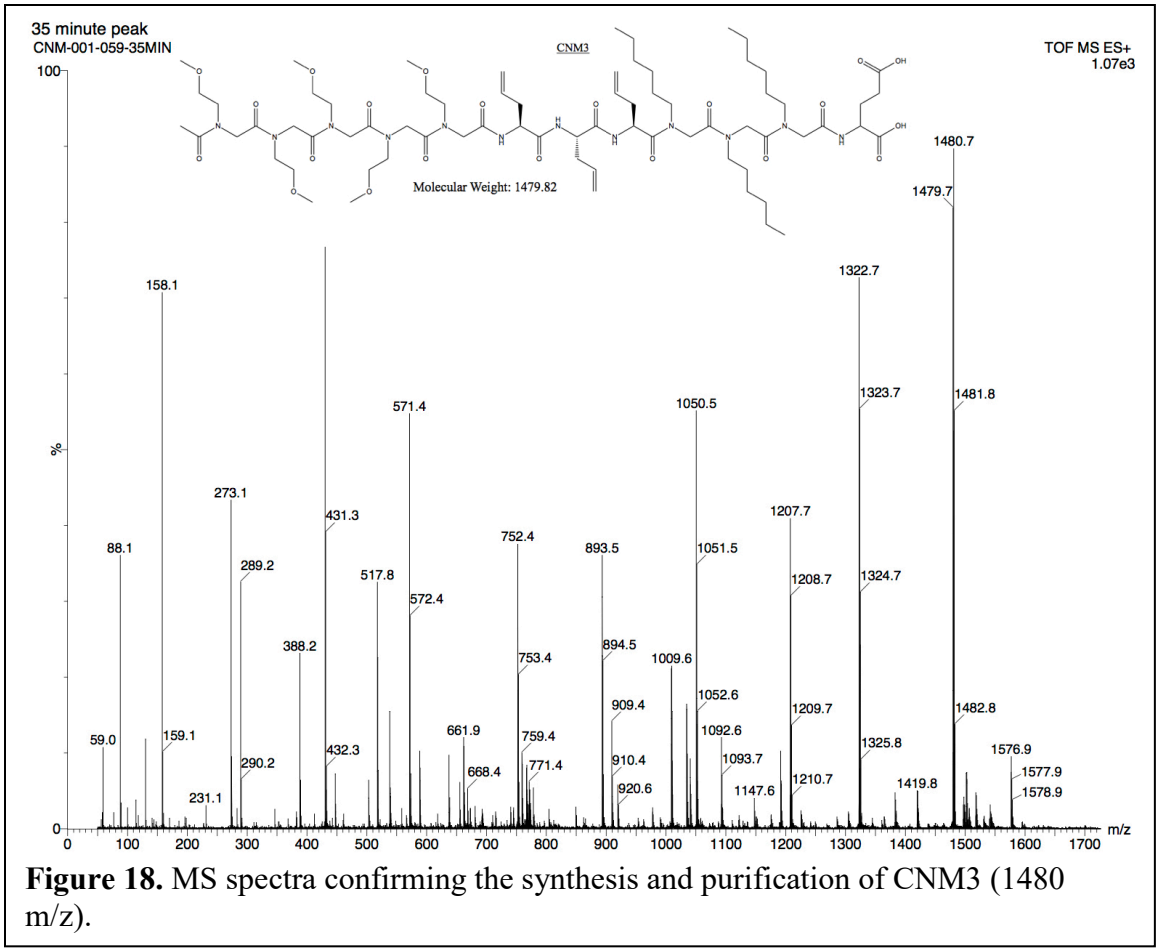

Figure 18. MS spectra confirming the synthesis and purification of CNM3 (1480 m/z).

The third and final derivative of CNM1, termed CNM4, resembled the structure of CNM2. However, it incorporated norbornene groups in the cross-linking moieties section instead of the allyl glycine groups used in the CNM2 and CNM3 cross-linking sections (**Figure 15**). CNM4 was synthesized using previously described methods, using the peptoid submonomer method and 5-norbornene-2-methylamine to install the norbornene moieties in the cross-linking section of the peptoid. CNM4 was cleaved and purified as described previously. However, an unexpected complication occurred during cleavage that led to a negligible yield with < 1 mg (< 0.3%) of purified product. The standard 95% Trifluoroacetic acid (TFA), 2.5% H_2O, and 2.5% triisopropylsilane (TIS) solution was used

to cleave CNM4 and had proven to be a reliable solution for previously synthesized molecules. However, when cleavage was performed with CNM4, the alkenes of the Norbornene submonomers in CNM4 underwent hydration to form alcohols. An M+1 peak at 1468 m/z was observed in the mass spectrometry results for the CNM4 sample which indicated that the structure was successfully synthesized (**Figure 19**). But the increasingly hydrated derivatives of CNM4 were also observed at 1486 m/z (one hydration), 1504 m/z (two hydrations), and 1522 m/z (three hydrations). Unfortunately, these hydrated derivatives made up a majority of the cleaved product from the original CNM4 synthesis. Because of this, all of the aforementioned steps for CNM4 synthesis were repeated, and a water-free solution of 97.5% TFA and 2.5% TIS was used to cleave CNM4. This time, synthesis and cleavage of CNM4 successfully yielded 14.7 mg (4.6%).

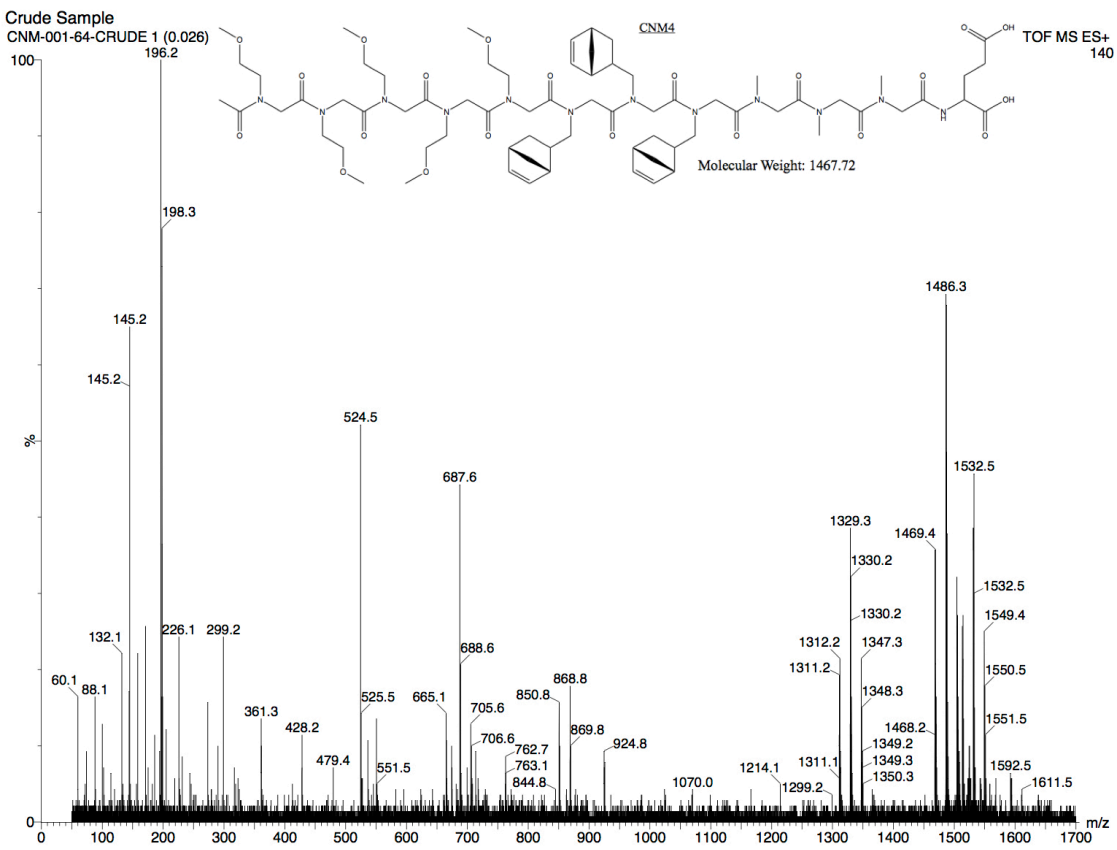

Figure 19. MS spectra confirming the synthesis and purification of CNM4 (1468 m/z).

Toxicity Testing of Full-Length Peptoids

The cytotoxicity of each of the three full-length peptoid variants (CNM2, CNM3, CNM4) was assessed following previously established protocols for HepG2 hepatocellular carcinoma cell testing.[14,15] The growth medium used to culture the HepG2 cells was a combination of several elements, but the base component was Dulbecco's Modified Eagle Medium (DMEM). Fetal bovine serum (FBS) made up 10% of the composite growth medium while another 1% consisted of penicillin/streptomycin/glutamine (PSG). The HepG2 cells were grown in composite growth medium loaded in T-75 flasks that were incubated at 37°C and 5% CO_2. After cell growth reached the desired concentration

between 1×10^5–4×10^5 cells/mL, the cells were isolated and resuspended in phenol red-free DMEM that contained 10% FBS and 1% PSG. The resuspended cells were seeded into 96-well plates and the plates were incubated for 1-2 hours at 37°C and 5% CO_2 so the cells could adhere to the plate. Using part of the purified samples from each of the full-length peptoid variants (CNM2, CNM3, and CNM4), 2-fold serial dilutions of 10x stocks of the desired final concentrations were prepared in water. The stock solutions were added in triplicates to the 96-well plates and the plates were incubated for 72 hours at 37°C and 5% CO_2. After the 72-hour incubation period, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) in water was added to each of the treatment wells before the plates were incubated for another 3 hours. Following this, the media was removed from the wells and dimethyl sulfoxide (DMSO, 100 μL) was added. The DMSO was added to lyse the cells and to solubilize any MTT that was metabolized by the cells. A SpectraMax M5 plate reader was used to measure absorbance at 570 nm so that percent inhibition could be subsequently determined. Triplicate assays for each of the three full-length peptoid variants (CNM2, CNM3, and CNM4) were all conducted twice on separate days to establish biological replicate studies for each sample.

Toxicity Levels of Full-Length Peptoids against HepG2 Cells

The toxicity level of each of the full-length peptoids (CNM2, CNM3, and CNM4) was assessed by measuring the amount of light absorbance that resulted after treatment with the various 10x stock concentrations. The measured light absorbance is based on the amount of metabolized MTT that is present in the wells of the test plate after the HepG2 cells have been lysed. A compound with a low toxicity will allow for a high level of cellular activity, meaning that a greater amount of MTT will be metabolized by the cells and a

darker shade of purple will be observed post-lysing. The values of light absorbance for the three compounds (CNM2, CNM3, CNM4) at each dosage were compared to the absorbances of the 0 μg/mL control group and the media control group in each of the assays. The comparison of the dosage absorbance levels to the absorbance levels of the controls provided percent inhibition values. These values were then converted into percent growth values that represent the level of HepG2 cell activity at each peptoid concentration. The three compounds were tested in triplicate at each concentration in at least two separate assays to collect two percent growth values. These values were used to calculate an average as well as a standard deviation for each dosage for all three compounds (**Figure 20**). A higher percent growth value indicates that the compound had a lower toxicity that allowed the HepG2 cells to grow. The average percent growth for all three compounds indicated that the peptoids were relatively non-toxic at concentrations at or below 100 μg/mL. This means that the compounds would need to be tested at levels higher than 100 μg/mL to determine the concentration at which the compounds become significantly toxic.

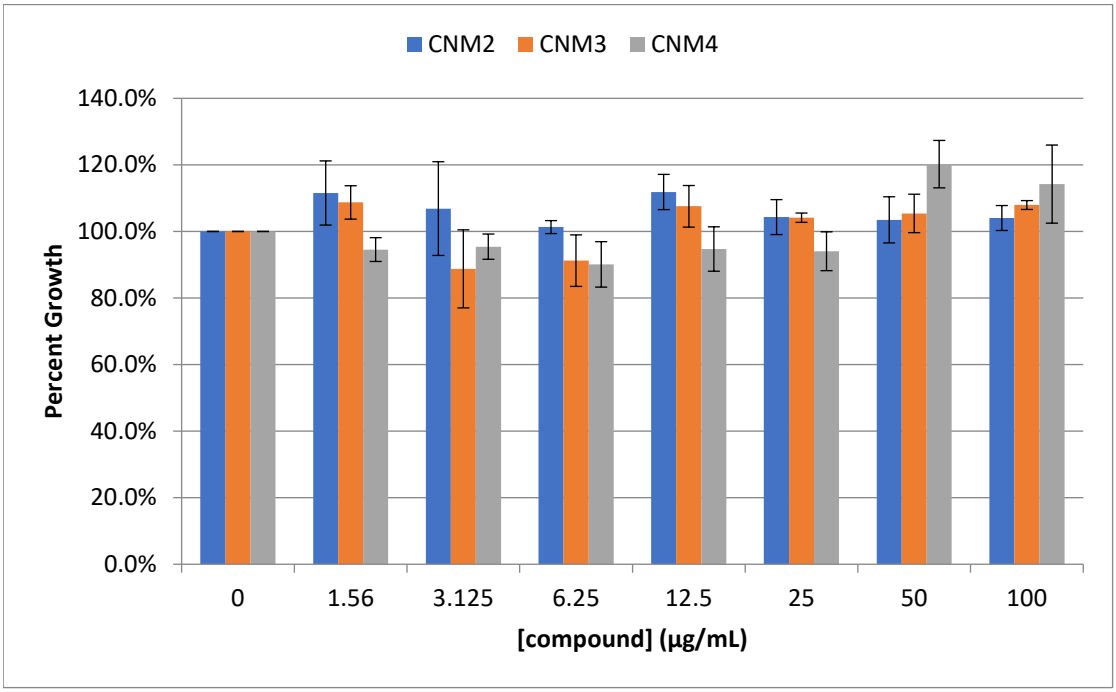

Figure 20. HepG2 hepatocellular carcinoma cell toxicity test results expressed as percent growth values for each of the three compounds (CNM2, CNM3, CNM4). The three compounds were tested at increasing concentrations from 0 µg/mL up to 100 µg/mL. The percent growth value for a compound at a certain concentrations represents the average of the values from two separate assays. The averages for each compound at each concentration were calculated in this way.

CONCLUSIONS AND OUTLOOK

The synthetic testing for QDP2 was useful in determining the ideal conditions to conduct reductive amination. It was found that reductive amination worked best when conducted in two phases starting with the addition of the aldehyde with an acid catalyst to a sample of resin. This mixture is reacted overnight at 35°C and washed the next day to remove remaining aldehyde. A solution of $NaBH_3CN$ is then added to the resin in the second phase and is left to react at 35°C for 3.5 hours. Conducting the reductive amination process in two phases under these specified conditions was determined to be the most effective method. This newly established method of reductive amination will serve as an

efficient synthetic scheme for adding submonomers with terminal alkenes to future peptoid derivatives.

The results from the coordination testing of QDP1 showed that peptoids with a C-terminal dicarboxylate could effectively coordinate to quantum dots. However, the poor yield from QDP1 synthesis indicated that a new coordination site was needed. Testing the coordination efficacy of QDP3 established that glutamic acid loaded Wang resin with the dianionic dicarboxylate already bound to the resin was an effective replacement for the QDP1 coordination site. This Wang resin was used to synthesize all subsequent peptoid derivatives in this research, and the resin will continue to serve as a reliable building block with an effective coordination site for future designed peptoids.

Coordination testing using QDP4, the hydrophobic derivative of QDP3, will be conducted in the future to assess the possible effects that a higher level of hydrophobicity might have on coordination efficacy. The synthesis of QDP5, the most hydrophobic derivative of QDP3, was unsuccessful and produced a yield that was too low to be used for coordination testing. It was observed that the synthetic yield for each peptoid steadily decreased as hydrophobicity increased from QDP3 to QDP4 to QDP5. This could indicate that the decreasing efficiency is correlated to unknown complications arising from the increasing hydrophobicity. A larger starting amount of Wang resin might be needed to conduct future synthesis of peptoids that incorporate submonomers with higher levels of hydrophobicity.

The HepG2 cell toxicity assays of CNM2, CNM3, and CNM4 all indicated that the designed peptoid derivatives had inherently low cytotoxicity levels. This was expected as peptoids mimic the structure and function of peptides that occur naturally in the human

body. These promising toxicity results are an indication that if the proposed peptoids can successfully coordinate and encase quantum dots, then the combined structures might be relatively biocompatible. The first step of future testing involving these peptoids will center around ensuring that the full-length derivatives can effectively coordinate to quantum dots. If coordination is successful, then research can move onto testing various polymerization methods that could be used to encase a quantum dot. Future research into this process for CNM2 and CNM3 will start with literature precedent that uses azobisisobutyronitrile (AIBN) as an initiator species and divinylbenzene (DVB) and styrene as the secondary compounds that will later react with the terminal alkenes of the cross-linking moieties.[16] Polymerization of norbornene containing CNM4 will be done using Ring Opening Metathesis Polymerization (ROMP) with Grubb's second generation catalyst, as previously reported.[17] Optimal polymerization conditions, including solvent, temperature, and initiating species will be determined in future research.

Once the most efficient method of polymerization is established and a complete shell can be formed around quantum dots, research will focus on testing the biocompatibility of the coordinated peptoid-quantum dot structures. The proposed full-length peptoids (CNM2, CNM3, and CNM4) exhibited low cytotoxicity levels, so if these peptoids can coordinate to quantum dots, encapsulate the toxic materials of the dots, and facilitate solubility in water, then the combined structures could be biocompatible enough for use in future biomedical applications.

REFERENCES

1. Yu, W. W., Chang, E., Drezek, R. & Colvin, V. L. Water-soluble quantum dots for biomedical applications. *Biochem. Biophys. Res. Commun.* (2006). doi:10.1016/j.bbrc.2006.07.160
2. Granada-Ramírez, D. A. *et al.* Quantum dots for biomedical applications. in *Nanobiomaterials* 411–436 (Elsevier, 2018). doi:10.1016/B978-0-08-100716-7.00016-7
3. Iancu, C. *et al.* Quantum dots in imaging, drug delivery and sensor applications. *Int. J. Nanomedicine* (2017). doi:10.2147/ijn.s138624
4. Iyer, G. *et al.* Peptide coated quantum dots for biological applications. *IEEE Trans. Nanobioscience* **5**, 231–8 (2006).
5. Caruthers, S. D., Wickline, S. A. & Lanza, G. M. Nanotechnological applications in medicine. *Curr. Opin. Biotechnol.* **18**, 26–30 (2007).
6. Chan, W. C. W. & Nie, S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science (80-.).* **281**, 2016–2018 (1998).
7. Wang, G., Li, Z. & Ma, N. Next-Generation DNA-Functionalized Quantum Dots as Biological Sensors. *ACS Chem. Biol* **13**, 59 (2018).
8. Allocca, M. *et al.* An Integrated Multilevel Analysis Profiling Biosafety and Toxicity Induced by Indium- and Cadmium-Based Quantum Dots in Vivo. *Environ. Sci. Technol.* **53**, 3938–3947 (2019).
9. Lau, J. L. & Dunn, M. K. Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorg. Med. Chem.* **26**, 2700–2707 (2018).

10. Rao, M. B., Tanksale, A. M., Ghatge, M. S. & Deshpande, V. V. *Molecular and Biotechnological Aspects of Microbial Proteases*. *Microbiol. Mol. Biol. Rev.* **62**, (1998).

11. Wolf, L. M., Servoss, S. L. & Moss, M. A. *Peptoids: Emerging Therapeutics for Neurodegeneration*. *J Neurol Neuromedicine* **2**, (2017).

12. Zuckermann, R. N., Kerr, J. M., Kent, S. B. H. & Moos, W. H. Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **114**, 10646–10647 (1992).

13. Amblard, M., Fehrentz, J.-A., Martinez, J. & Subra, G. Methods and protocols of modern solid phase peptide synthesis. *Mol. Biotechnol.* **33**, 239–254 (2006).

14. Spicer, S. K. *et al.* Toward a clinical antifungal peptoid: Investigations into the therapeutic potential of AEC5. *Biopolymers* **110**, e23276 (2019).

15. Green, R. M. & Bicker, K. L. Evaluation of Peptoid Mimics of Short, Lipophilic Peptide Antimicrobials. *Int. J. Antimicrob. Agents* **56**, 106048 (2020).

16. Castaldo, R. *et al.* Synthesis and adsorption study of hyper-crosslinked styrene-based nanocomposites containing multi-walled carbon nanotubes. *RSC Adv.* **7**, 6865–6874 (2017).

17. Skaff, H. *et al.* Crosslinked Capsules of Quantum Dots by Interfacial Assembly and Ligand Crosslinking. *Adv. Mater.* **17**, 2082–2086 (2005).