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Yusheng Eric Zhang April 13, 2015

Synthesis of Diphenylalanine-based Peptide Aldehydes and their Self-Assembly Propensity in a Dynamic Combinatorial Library

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Abstract

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By Yusheng Eric Zhang

Small peptides with the ability to self-assemble have been investigated both for novel biomaterial discovery purposes and for origins of life research. Diphenylalanine is an example of a small peptide that have been thoroughly studied, and it has been shown to exhibit diverse self-assembly capabilities. Furthermore, chemical modifications that allow each peptide monomer to dynamically link with one another gives the peptide systems even greater possibility to form diverse structures. Inspired by previous studies in the Lynn Lab, this paper discloses the synthesis of diphenylalanine-based peptide aldehydes and discusses the various attributes of the dynamic combinatorial library (DCL) established using the diphenylalanine based peptide aldehydes.

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Introduction

Proteins' importance in biological systems cannot be disputed; as a result, extensive effort has been put into engineering proteins to study, to alter, or even to create novel biological functions. Going deeper into the context of biological systems, peptides also play important roles ranging from signal displays to hormones (oxytocin, growth hormone, follicle-stimulating hormone, etc). Even more interesting is the potential for peptides, in particular small peptides with the capability to self-assemble, to be exploited for a wide variety of purposes. These may range from biomaterial engineering to explorations in chemical evolution.

Molecular self-assembly, which is a process that is mediated by weak intermolecular forces such as hydrogen bonding, hydrophobic interactions, etc. has been described as "a powerful approach for fabricating novel supramolecular architectures."[1] Adhering to these basic understandings, peptides have emerged as a popular option in constructing these supramolecular structures. Previous experiments in this field have used a histidine-rich peptide scaffold for the fabrication of nanowires by coating the tubes with not only gold, but also with avidin, which gives these tubes the ability to bind biotinylated as well as other diverse surfaces.[2][3] In addition to guiding the construction of inorganic materials, peptide-based building blocks have also been used for the purpose of tissue regeneration. A peptide-amphiphile has been demonstrated to self-assemble into a fibrous scaffold that directed hydroxyapatite mineralization; the results of this mineralization process produced a composite material that highly resembles bone tissues.[4] Other projects have attached peptide signaling sequences onto self-assembling peptides to generate a 3-D environment that encouraged selective differentiation of neural progenitor stem cells.[5] These early successes demonstrate the potential of peptides and peptide-based materials to serve as excellent building blocks of novel biomaterials.

In addition to designing novel biomaterials, current peptide research also has a context in chemical evolution and origins of life research. Chemical evolution undoubtedly preceded biological evolution, and the earliest players in chemical evolution must have the qualities to store coded information, catalyze reactions, as well as self-replicate.[6] RNA fits this mold as it is widely known to have the ability to self-replicate and to form enzyme-like catalysts, let alone store coded information. However, from a chemist's perspective, RNA is unlikely the first players in chemical evolution. Nucleotides are complicated molecules that are unlikely to be spontaneously synthesized in the open and chaotic environment of prebiotic earth.[7][8] Furthermore, side products (i.e. enantiomers) are likely to be formed in any chemical reactions, especially uncontrolled ones. An experiment from 1984 determined that the oligomerisation of a growing RNA chain on a poly(C) template is inhibited by the incorporation of RNA's enantiomer by chain termination.[9] All of the above reasoning and experimental evidence suggests that chemical evolution likely began with simple, chiral molecules, and chiral encoding has been suggested as a possible solution to answering questions about the origin of life.[6]

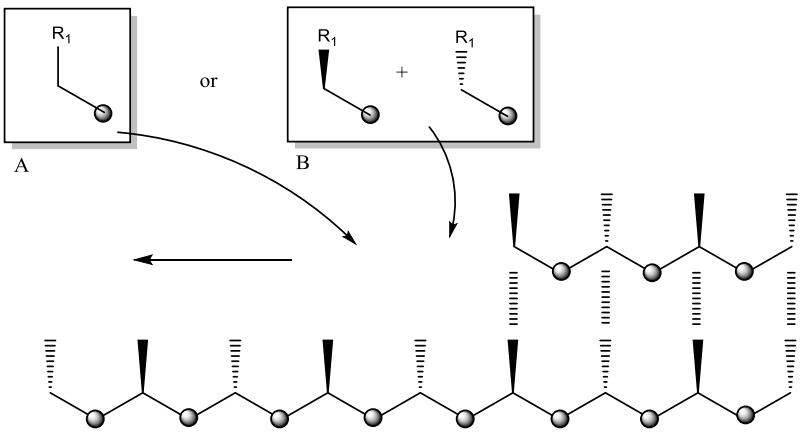


Figure 1. Illustration of chiral encoding. Achiral monomers (a) or simple enantiomers (b) polymerize to form stereoisomeric products, which in turn serve as templates for generating more copies of itself. Figure adapted from [3].

The principle of chiral encoding is rather simple: achiral monomers (with no stereocenters) or pairs of simple enantiomers may polymerize to give stereoisomeric products

that serve as templates for making more copies of itself from the monomeric building blocks (figure 1).[6] Some examples of such simple enantiomers include peptides, as many of which only have one stereocenter at the alpha carbon. Recent experiments have used short amphiphilic β-sheet forming peptides to demonstrate self-replication, which ultimately lead to self-assembly.[10] These peptides were chemically modified so that different monomeric peptides auto-catalyze the irreversible native chemical ligation process creates a longer monomeric product (figure 2). These monomeric products in turn aggregate and serve as templates for further autocatalysis as the kinetics of this process increases with time.

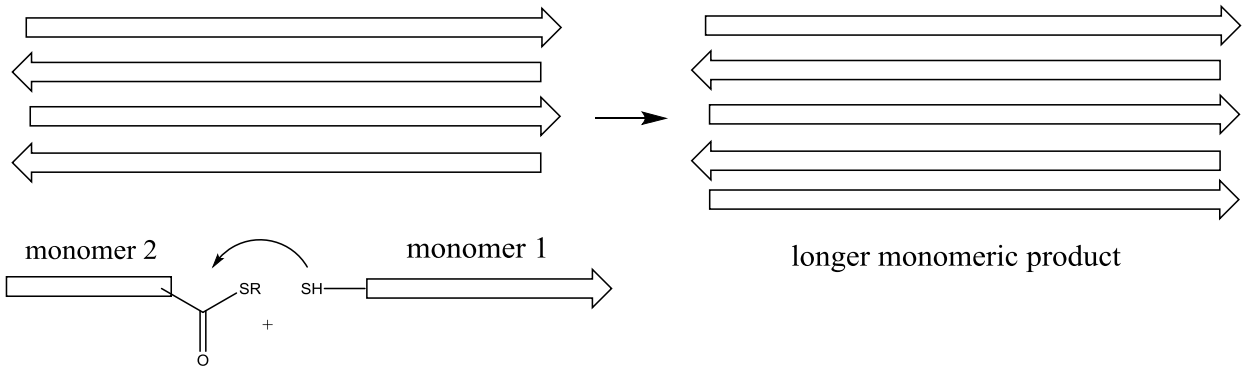


Figure 2. The antiparallel β sheet is formed from the longer monomeric products, which in turn serves as a template to guide its self-replication. Two monomers are guided by this template to undergo native chemical ligation and form longer monomeric products. Figure adapted from [10].

Even more interesting is the use of dynamic combinatorial chemistry (DCC) in the investigation of non-RNA molecules in evolution and self-replication. DCC "rests on the design and the study of libraries of species connected by reversible (supra)molecular bonds."[11] This would give the chemical system an additional dimension of possibility for change since reversible bonds are prone to the slightest alterations in the environment. Thus, the abundance of each chemical species in the chemical system is prone to environmental changes; a new chemical species may replace another as the predominant species in the chemical system.[12] In a recent study, a dynamic combinatorial library (DCL) was constructed with one hydrophobic component

expressing an aldehyde functional group and several hydrophilic components expressing various amine groups (primary amine, benzyl amine, aniline, hydroxylamine, etc) (figure 3).[11] Experiments within this study not only provided evidence of self-replication, it also demonstrated that with changes in the environment (switching from organic solvent to aqueous solvent), the predominant imine formed with the hydrophobic aldehyde switched from that of the primary amine to that of the aniline.[11]

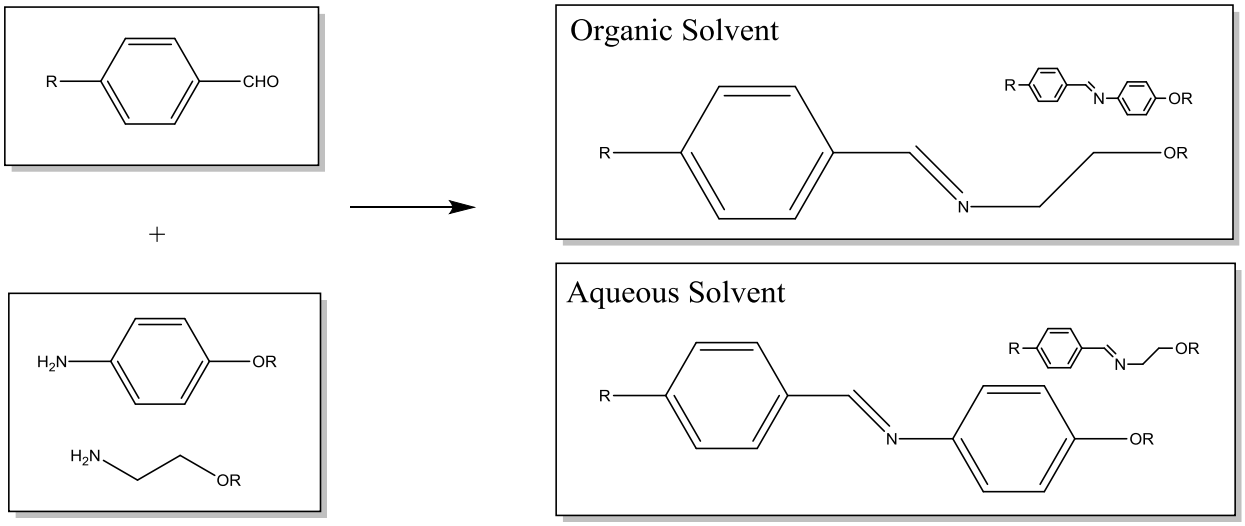
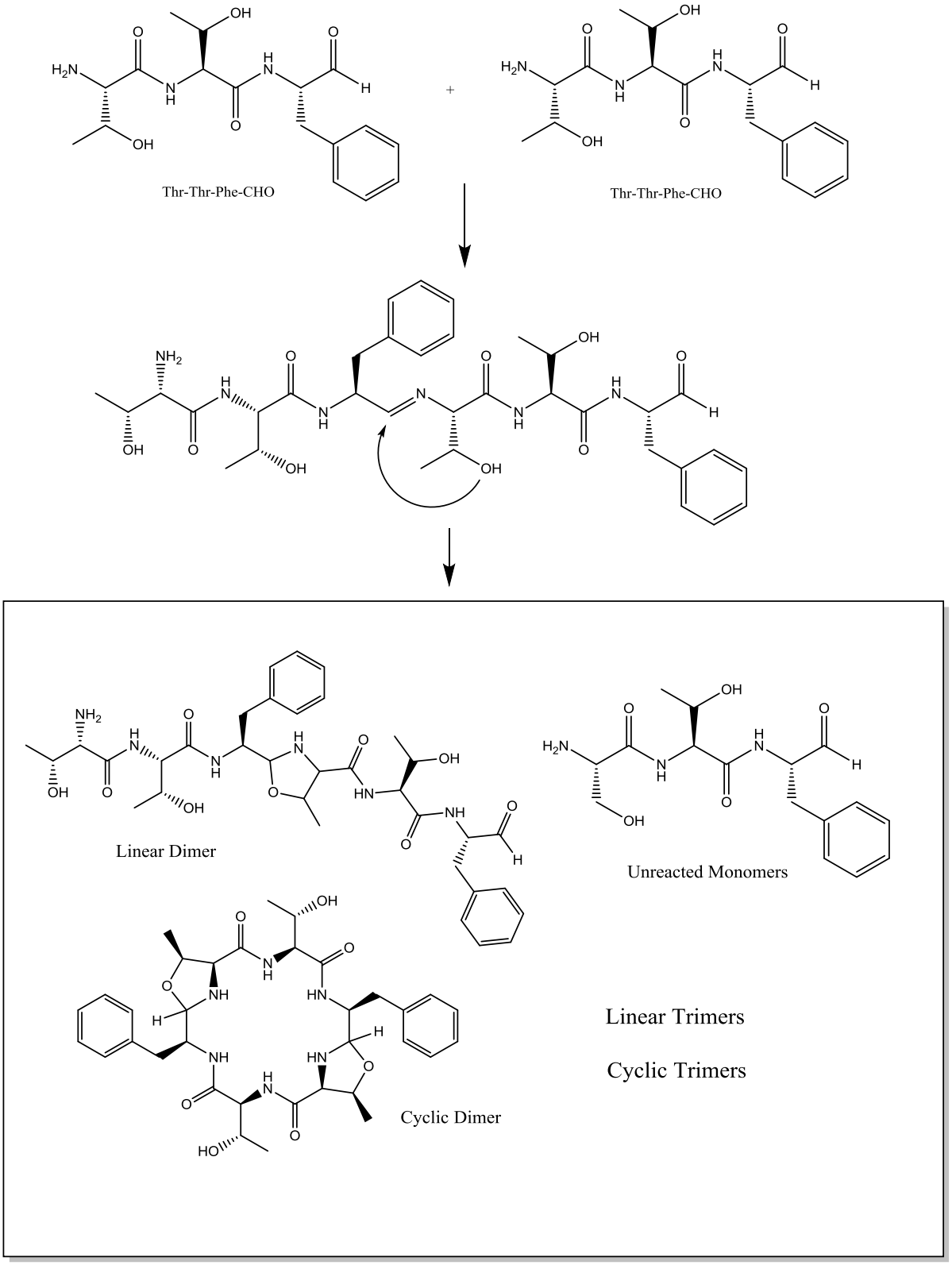


Figure 3. The various amines (aniline or primary amine) compete for the aldehyde group. The predominant chemical species in each chemical environment is illustrated by the relative size of the molecule in each environmental condition. Figure adapted from [11].

However, the supramolecular structures observed so far in both of these disclosed experiments are limited to micelles. Previous work in the Lynn lab demonstrated that a peptide sequence derived from amyloid β proteins is capable of ultimately forming tubular structures. Inspired by this observation as well as by data from recent virtual screening for the self-assembly propensity of various dipeptide sequences,[13] my former mentor Dr. Junjun Tan began investigation the self-assembling capability of the L-thr-L-phe based peptide aldehydes (-TF-CHO) in a dynamic combinatorial library.[14]



Scheme 1. Within a DCL constructed of TTF-aldehydes, two TTF-aldehyde monomers may polymerize at first via imine formation followed by the closing of the ring structure to form an N,O-acetal linkage. In the box are examples of some of the possible chemical species that constitute this DCL. Figure adapted from [14].

The dynamic covalent chemistry occurs as thus: the N-terminal amine attacks the C-terminal aldehyde either intra-molecularly or intermolecularly and forms an imine intermediate; this is followed by an attack from the hydroxyl group on the side chain of Thr, which forms the linear dimer/cyclic dimer with heterocyclic N,O-acetal linkage (scheme 1). This investigation began for the ultimate purpose of both discovering an ideal chemical system to investigate characteristics of chemical evolution as well as designing peptide based building blocks that can serve as dynamic biomaterials. Besides linear and cyclic dimer products in the TTF-CHO dynamic network, there was a slight presence of trimers, although this trimeric specie did not predominate. It was also discovered that for threonine-phenylalanine based peptide aldehydes, having a significant trimeric presence is crucial for the formation of more diverse supramolecular structures (nanotubes in particular) as well as their self-replication. We thought that the methyl group on threonine's side chain may have hindered the formation of the cyclic N,O-acetal linkage; therefore, my mentor and I began synthesizing the STF-aldehyde sequence and investigating the chemical network established from this sequence. Unfortunately, recent experiments using the STF-aldehyde sequence also did not achieve a predominant trimeric presence nor much diversity in self-assembly.

Unlike the threonine-phenylalanine based peptide aldehyde sequences, diphenylalanine peptides (unmodified and without the ability to reversibly link) have been extensively studied both in laboratory settings and in computer simulations.[15][16] It is known that L-Phe-L-Phe forms tubular and wire structures in aqueous environments and can be manipulated to form other structures via alterations in solvent system, pH value, and temperature.[16][17][18] Furthermore, in molecular simulations, altering the peptide concentration in a diphenylalanine system can lead to different final structures from nanovesicles to nanotubes to bilayers.[15] I initially decided to use

diphenylalanine-based peptide aldehydes instead of threonine-phenylalanine based peptide aldehydes in subsequent experiments because of its comparative synthetic simplicity (explained in part 1). However, upon further consideration, it seemed possible that the increased hydrophobicity of an additional phenylalanine in a peptide aldehyde monomer may enhance its propensity to aggregate and form longer polymers. This may very well result in the establishment of a DCL in which trimeric species predominate. Therefore, it was determined that one of the next sequences to be investigated would be SFF-aldehyde.

Furthermore, the dynamic covalent linkage formed between two monomers is in fact cyclic. Both threonine and serine form five-membered rings after the initial formation of the imine and the subsequent attack of the hydroxyl side chain. However, it is widely believed that the formation of six-membered rings is more favorable than that of five-membered rings because the extra member in the ring would provide enough room for each bond to attain their ideal sp^3 bond angle. Therefore, it is believed that in addition to the usage of a diphenylalanine-based sequence, installing an L-homoserine would encourage the formation of trimeric (or even longer) species in our DCL because homoserine's side chain would result in the formation of a six-membered ring, since it is one methylene group longer than the serine's side chain (figure 4). Therefore, the sequence hSFF-aldehyde was also determined to be investigated.

Once again, the ultimate purpose of this area of research lies in the improvement of biomaterial design and in the investigation of chemical evolution. The purpose of my particular project is to investigate chemical qualities that may improve upon the peptide-aldehyde systems that we have previously investigated. The goal of my particular project entailed the synthesis, establishment, investigation, and comparison of the SFF-aldehyde and the hSFF-aldehyde DCLs. Furthermore, I intended on establishing a mixed SFF and hSFF-aldehyde library for the purpose

of providing a direct and exact comparison that contrasts the propensities of six and five-membered ring formation. This paper documents the process towards attaining my goal as well as the results from various experiments thus far.

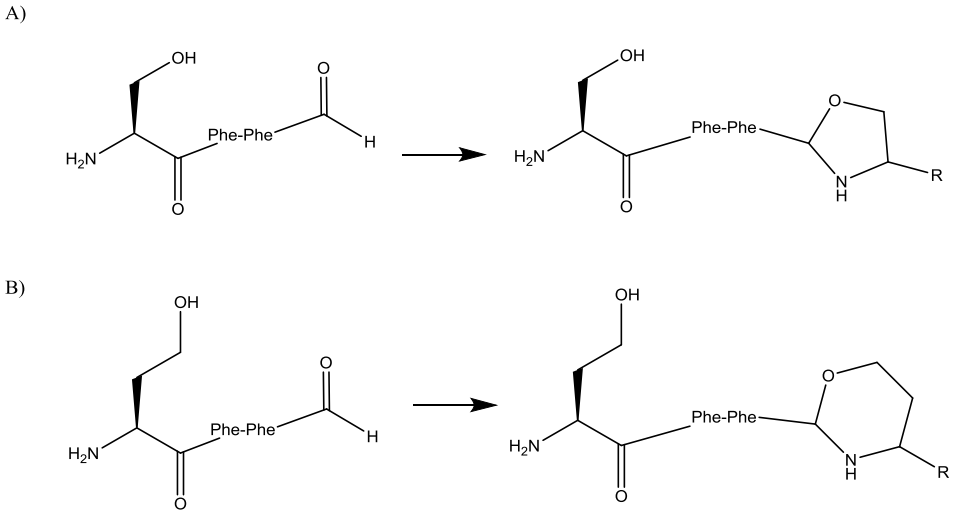


Figure 4. SFF-CHO (A) forms a five-membered reversible N,O-acetal ring when it polymerizes with another monomer. hSFF-CHO (B), on the other hand, forms a six-membered ring when it polymerizes.

Part 1: Synthesizing the Peptides

Coupling Amino Acids

12 grams of (a) Boc-L-Phe-OH was first modified to Boc-L-Phe-Weinreb amide with (b) 4.85 grams (1.1 mol. equiv.) of N,O-dimethylhydroxylamine · HCl and (c) 8.07 grams (1.1 mol. equiv.) of 1’1-Carbonyldiimidazole. This reaction was dissolved in 80 ml of dichloromethane (DCM). Reactant (a) and (c) were first dissolved in DCM and were allowed to activate for one hour. After activation, (b) was added and allowed to react overnight. The DCM was then removed in vacuo and the residue was re-dissolved in approximately 50 ml of EtOAc and washed successively with 1 M aqueous HCl, saturated aqueous $NaHCO_3$ solution, and brine. After drying with Na_2SO_4, the organic layer was removed in vacuo and the product was allowed to dry in a vacuum. The yield for this step was greater than 93% (scheme 2).

The next Phe was then coupled onto the Phe-Weinreb amide. The Boc group on the synthesized Boc-Phe-Weinreb amide was removed with 4 M HCl in dioxane solution for two hours. For this reaction, 13 grams of Boc-Phe-Weinreb amide and 60 ml of the dioxane HCl solution were used. The acidic solution was subsequently removed in vacuo, and the dried product was allowed to dry in vacuum for at least 24 hours. To couple on the next L-Phe, 2.50 grams of Phe-weinreb amide, 3.82 grams (1.2 mol. equiv.) of Boc-Phe-OH, 1.95 grams (1.2 mol. equiv.) of Hydroxybenzotriazole (HoBT), 2.24 grams (1.2 mol. equiv.) of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and 4.73 ml (3 mol. equiv.) of Triethylamine (Et_3N) were dissolved in 30 ml DCM. This was allowed to react overnight. The mixture was then dried in vacuo, and the residue was dissolved in EtOAc and processed with the previously discussed washing procedure before the organic layer was removed in vacuo. After drying in vacuum overnight, the Boc group was again removed with 4 M HCl in Dioxane for two hours. The acid solution was then removed in vacuo, and the product was allowed to dry in vacuum for at least 24 hours. The yield for this step was greater than 95%. Boc protected amino acids were used instead of Fmoc protected ones because of the ease in purification after coupling. Upon deprotection with acid, the removed Boc group collapses into carbon dioxide and tert-butylene that are removed in vacuo. However, the Fmoc protection group collapses into carbon dioxide and a bulky conjugated aromatic product upon deprotection that requires extensive efforts to remove (typically via extracting the deprotected peptide in acidic aqueous solution from organic layer). Since phenylalanines are incredibly hydrophobic, there were doubts that a diphenylalanine could be extracted with acidic aqueous solution from EtOAc.

To couple on the L-serine or the L-homoserine, Fmoc protected amino acids were used. To make Fmoc-SFF-Weinreb amide, 2.62 grams (1.2 mol. equiv.) of Fmoc-S(t-butyl)-OH, 2.23

grams (1 mol. equiv.) of Cl^- FF-Weinreb amide, 0.92 grams (1.2 mol. equiv.) of HoBT, 1.06 (1.2 mol. equiv.) of EDC, and 2.69 ml (3 mol. equiv.) of Et_3N were dissolved in 30 ml of DCM and allowed to react for 4.5 hours. The DCM was removed in vacuo, the residue was then processed the same way as previously, and it was allowed to dry in vacuum overnight. The t-butyl group on the Fmoc-S(t-butyl)FF-Weinreb amide was removed overnight with pure trifluoroacetic acid (TFA), at which point the acid was removed in vacuo and the product was dried in vacuum for at least 48 hours. Typically in coupling reactions that use Fmoc protected amino acids, there are many unknown side products that are produced. Furthermore, there are typically some Fmoc groups that are deprotected in the process (the resulting product has a blue glow under short-length UV light as visualized on TLC plates). To purify the Fmoc-SFF-Weinreb amide, a normal phase flash column chromatography was conducted using silica gel and an EtOAc/hexane mixture as the elution solvent. The chromatography generally begins at 70% hexane and 30% EtOAc, and the proportion of EtOAc increases gradually. The Fmoc-SFF-Weinreb amide peptide elutes at 20% hexane and 80% EtOAc. The fractions containing the peptide was collected, dried in vacuo, and stored. The same procedure was repeated to synthesize Fmoc-TFF-Weinreb amide, which will be used for future experiments. The yield for Fmoc-SFF-weinreb amide was around 30%; the yield for Fmoc-TFF-weinreb amide was around 50%.

Efforts in Procuring Homoserine

Fmoc-hSer(trityl)-OH, the fully protected L-homoserine, is an expensive reagent. Therefore, it was decided to purchase the L-hSer-OH and to manually protect its N-terminal amine and its side chain. The homoserine was first protected with an Fmoc group. 1.00 grams of hSer-OH and 2.61 grams (1.2 mol. equiv.) of Fmoc-Cl were first dissolved in 20 ml of Dioxane. 40 ml of 10% $NaHCO_3$ was slowly added to the reaction mixture, and the reaction was allowed

to proceed at room temperature overnight. The dioxane was then removed in vacuo, and the aqueous mixture was washed with diethylether (EtOEt) two to three times (30 ml each time). The mixture was then acidified with HCl until Fmoc-hS-OH began precipitating, taking care not to create spots of extreme acidity in the mixture (previous reactions in which the mixture was too acidic resulted in self-esterification of the homoserine). The Fmoc-hS-OH was then extracted with EtOAc, dried with Na_2SO_4, and dried in vacuum overnight. The yield of this step is generally greater than 95%.

To protect the side chain of the homoserine, 1.5 grams of Fmoc-hS-OH was allowed to react overnight with 1.47 grams (1.2 mol. equiv.) of Trityl Chloride and 0.79 ml (2.2 mol. equiv.) of anhydrous pyridine in 30 ml DCM. In order to purify the newly made Fmoc-hS(trt)-OH, a column chromatography was attempted. However, the product mixture would not dissolve in EtOAc or DCM, even upon sonication. Therefore, extraction from EtOAc with a basic solution seemed like the next best option. After extraction, the basic solution was acidified, and the product was extracted again with EtOAc, dried with Na_2SO_4, and stored in vacuum. However, very little product was isolated using this process, and the vast majority of what was isolated was in fact not the desired product: Fmoc-hS(trt)-OH. The protection procedure was attempted again. After waiting more than a month for both Fmoc-Cl and Trityl-Cl to arrive, this protection procedure was repeated again, except column chromatography was attempted in the same day without drying the product. However, upon addition of small quantities of hexane, white solids began to precipitate out of the solution as before. Fortunately, TLC revealed that the white solids were in fact not the protected amino acid. Therefore, removal of the solids was done prior to performing another chromatography. Suspiciously, the amount of white solids removed from the mixture appeared almost equal the amount of trityl chloride initially used for the protection

reaction. Upon further consultation with other graduate students, it seemed that literature values for this protection reaction's yield are not very high. Despite the time and effort spent into protecting the homoserine, a batch of the fully protected homoserine was found online from an American vendor for a reasonable price and was subsequently purchased. In total, this whole attempt took more than two months.

Synthesis of Fmoc-hS(trt)-FF-Weinreb amide was first attempted in a small batch. 0.2 grams of Fmoc-hS(trt)-OH was coupled with 0.16 grams (1.2 mol. equiv.) of Cl^- FF-weinreb amide using 0.064 grams (1.2 mol. equiv.) of EDC, 0.055 (1.2 mol. equiv.) of HoBT, and 0.11 ml (3 mol. equiv.) of Et_3N in 8 ml of DCM. After reacting for 5 hours, the reaction was processed using the same previous procedure. After drying in vacuum overnight, the trityl group was removed in a 50% TFA solution in DCM for 1.5 hours. The TFA/DCM solution was removed in vacuo, the residue was dried in vacuum for at least 24 hours, and a normal phase column chromatography was conducted using the same parameters as before. The leftover trityl side product eluted around 50% hexane/50% EtOAc while the peptide product eluted around 25% hexane/75% EtOAc. The fractions containing the product were collected and dried in vacuo, and the product was stored in vacuum for at least 24 hours. An NMR experiment confirmed the achievement of the Fmoc-hSFF-Weinreb amide peptide as both the Fmoc group (which appears around 7 to 8 ppm) and the Weinreb amide group (two solitary peaks between 3 to 4 ppm) appeared. TLC revealed no presence of the side product of Fmoc removal. The final yield of this reaction was slightly less than 30%. This coupling reaction was attempted on and was successful with TF-Weinreb amide as well.

The Fmoc-hS(trt)-OH coupling reaction was then scaled up, and two batches of Fmoc-hSFF-Weinreb amide were synthesized and purified separately; from now on, the first batch will

be referred to as batch (a) and the second will be referred to as batch (b). Fmoc-SFF-Weinreb amide and Fmoc-TFF-Weinreb amide were also made separately in large quantities. At this point, all three peptides were ready to be converted into peptide aldehydes.

Synthesis, purification, and protection of peptide aldehydes

Installation of the Weinreb amide on the C-terminus of the peptide allows for preferential reduction of this particular carbonyl group into an aldehyde. However, with an acidic proton on each amide nitrogen and with the side chains of the homoserine/serine/threonine unprotected, the ideal ratio of reductant to peptide is no longer one to one and had to be optimized. The reductant used was lithium aluminum hydride ($LiAlH_4$), the overuse of which has been shown to reduce the aldehyde further into a hydroxyl group on a similar peptide. The first attempt at reduction was conducted on the original small batch of Fmoc-hSFF-Weinreb amide. One mol. equiv. of the peptide (0.07 g) was allowed to react with 1.5 mol. equiv. of $LiAlH_4$ in 2.5 ml tetrahydrofuran (THF). The peptide was first dissolved in THF; after the peptide completely dissolved, the reaction mixture was cooled and kept at -78℃ with a dry ice acetone bath. The $LiAlH_4$ was inserted at this temperature, and after insertion, the reaction mixture was allowed to react at 0℃ using an ice bath for 25 minutes. After the reaction was complete, the reaction was quenched with 1 M $KHSO_4$ solution. The amount of the bisulfate solution used was 10 times the volume of the reductant used. The THF was subsequently removed in vacuo, and the peptide aldehyde was extracted with EtOAc and washed with saturated $NaHCO_3$ solution. The product was stored in vacuum overnight; the appearance of a peak around 9.5 ppm in an NMR experiment confirmed the presence and success of the reduction. Without purifying the peptide aldehyde, the mixture was dissolved in 5.00 ml of 1.25 M HCl in methanol solution and was allowed to react under constant reflux at 65℃ (methanol's boiling point) for 15 minutes to convert the aldehyde group

into an acetal so that the product can be stably kept for longer periods of time. The acidic methanol solution was then removed in vacuo, and the resulting residue was stored in vacuum overnight.

This same exact procedure was scaled up and repeated on batch (a), and the product of the large batch reduction was combined with that of the small batch reduction. To isolate the desired peptide acetal, a column chromatography was again conducted on the resulting reaction mixture, except alumina gel was used instead because the acetal group on the peptide aldehyde is sensitive to the acidity of the silica gel. However, when attempting to dissolve the product for chromatography in the usual EtOAc/hexane solvent, a white substance began precipitating out of the solution. Regardless of this observation, half of the precipitating mixture (a1) was inserted into the column to begin the chromatography, and half (a2) was dried in vacuo and stored in vacuum. However, regardless of what solvent (DCM, 100% EtOAc, even methanol) was used, the precipitant stayed at the very top of the column and clogged the chromatography process. The only eluent observed were some detached Fmoc protecting group, which glowed with a blue hue under short length UV light on silica TLC. Furthermore, a TLC run revealed that the mixture is incredibly messy. Therefore, after losing batch (a1) to the column, it was hypothesized that purification via chromatography must occur immediately after the reduction. Thus, attention was turned to batch (b).

Batch (b) was reduced, extracted, and stored using the same method as stated above except for converting the aldehyde into an acetal using 1.25 M HCl in methanol. A chromatography was attempted on batch (b); however, a white substance continued to precipitate out of the solution even when it was initially dissolved in 100% EtOAc. It was then decided that the precipitant must be first removed from the solution and investigated after purification.

Therefore, the precipitant was filtered out. The amount of white precipitate filtered from the mixture was astounding, so a TLC was conducted to investigate the possible identity of this substance. Upon examining the TLC plate, it was revealed that the substance was very pure with very few impurities. A side-by-side TLC run with both Fmoc-hSFF-Weinreb amide and the white substance showed co-elution. It was then decided to isolate as much precipitate as possible. The filtrated solution was then dried in vacuo, the residue was then dissolved in minimal amount of EtOAc, and hexane was slowly added until the precipitant appeared and could be filtered. The process was repeated until no more precipitant appears. After combining all the white precipitant, dissolving them with acetone, drying the mixture in vacuo, and then storing the product in vacuum for at least 24 hours, a subsequent NMR study was conducted and revealed that the white precipitant was indeed the desired Fmoc-hSFF-aldehyde (figure s1). No trace of unreacted Fmoc-hSFF-Weinreb amide was detected in the NMR experiment. Batch (b) was then protected with an acetal group using the constant reflux procedure outlined previously.

Unwilling to discard batch (a2), another chromatography was attempted and thwarted by the same precipitating white substance. Therefore, using the same logic as above, the white substance was filtered and investigated using TLC. Fortunately, it was revealed that this filtered white substance co-eluted with mostly-pure Fmoc-hSFF-acetal (batch (b)). Thus using the same precipitation-inducing method outlined previously, maximum amounts of the white substance was isolated from the reaction mixture.

Fmoc-SFF-Weinreb amide and Fmoc-TFF-Weinreb amide were both reduced using 1.5 and 1.4 mol. equiv. of $LiAlH_4$ respectively in the same reduction procedure as outlined previously. Based on the previously discussed observations of the solubility of Fmoc-hSFF-aldehyde and Fmoc-hSFF-acetal, the newly synthesized Fmoc-SFF-aldehyde and Fmoc-TFF-

aldehyde were extracted, protected with the acetal group, and stored using the same procedure that was used on the homoserine counterpart. The precipitation-inducing procedure discussed previously proved to be an efficient way to isolate diphenylalanine-based peptide aldehydes from the reaction mixture. Except for a small amount of highly-polar impurities, the isolated product was largely the desired peptide aldehyde. Because of the presence of impurities, the percent yield for this step was not calculated. At that point, the three peptide products were Fmoc-SFF-acetal, Fmoc-TFF-acetal, and Fmoc-hSFF-acetal.

Fmoc removal and further purifications

Fmoc was removed from both the Fmoc-SFF-acetal and the Fmoc-hSFF-acetal using a 20% piperidine solution in acetonitrile. This deprotection reaction continued for 20 minutes, at which point the solution was removed in vacuo and the residue was stored in vacuum for at least three days to remove the leftover piperidine from the residue. After Fmoc removal, SFF-acetal and hSFF-acetal were dissolved in a deionized water/ether mixture, which was later allowed to separate in a separation funnel. The aqueous layer was isolated and washed two more times with ether to remove as much of the Fmoc as possible. After this was completed, the volume of the water layer was reduced in vacuo to less than 25 ml in volume, at which point it was rapidly frozen with liquid nitrogen and lyophilized overnight.

Acetonitrile and water are the two solvents typically used to elute peptides from the HPLC. Traditionally, both acetonitrile and water contain 0.4% TFA to provide an acidic pH. However, these two peptide acetals are not stable in acidic conditions. Therefore, all subsequent preparatory HPLC purifications and analytical HPLC experiments were conducted using aqueous 1 mM triethylammonium acetate (TEAA) buffer at pH 7 and pure acetonitrile. Before using the preparatory HPLCs, the columns are usually rinsed with pure water for 20 minutes in order to

remove all traces of TFA and salt. This was followed by rinsing the column with pure acetonitrile for 30 minutes in order to remove all other impurities in the column. Typically, the column is then re-equilibrated with the TEAA buffer system before the injection and purification of the peptide acetals.

In order to prepare the samples for injection, the lyophilized peptide acetals were initially dissolved in minimal amounts of TEAA buffer. Due to having two Phe residues, the aqueous buffer alone does not fully dissolve the peptide, so acetonitrile was slowly added to the mixture until all the peptides were dissolved. The mixture was then sonicated for 10 minutes before injection. The SFF-acetal peptide was purified using the Jasco HPLC system with a 19 by 250 mm Atlantis C_{18} 10μm prep column in the Lynn lab. An initial fast method of 10% to 60% acetonitrile in 50 minutes at a flow rate of 20 ml per minute revealed that the suspected SFF-acetal peptide eluted around 36% acetonitrile. Therefore, a new method, 20% to 50% acetonitrile in 30 minutes at a flow rate of 20 ml per minute, was implemented and used to purify the peptide mixture. The suspected SFF-acetal peptide eluted at 33.5% to 34% acetonitrile, and all the eluent containing the peptide were combined. Initial MALDI experiments conducted on the suspect SFF-acetal using the α-Cyano-4-hydroxycinnamic acid matrix (α-CHCA, 20 mg/ml in TFA buffer) were inconclusive, and it was hypothesized that the sample may be either too dilute (peptide m/z peak indistinguishable from background noise) or too unstable in the acidic matrix. Therefore, the total volume of the eluent was reduced to 20 ml in vacuo, at which point it was lyophilized. After lyophilization, a small portion of the dried peptide was dissolved in methanol and submitted for exact mass spectrometry. The single most prominent peak showed a mass over charge (m/z) value of 430, which is the exact mass of the SFF-acetal peptide (figure 5). At that point, the SFF-acetal peptide was determined to be pure and ready for use in establishing a DCL.

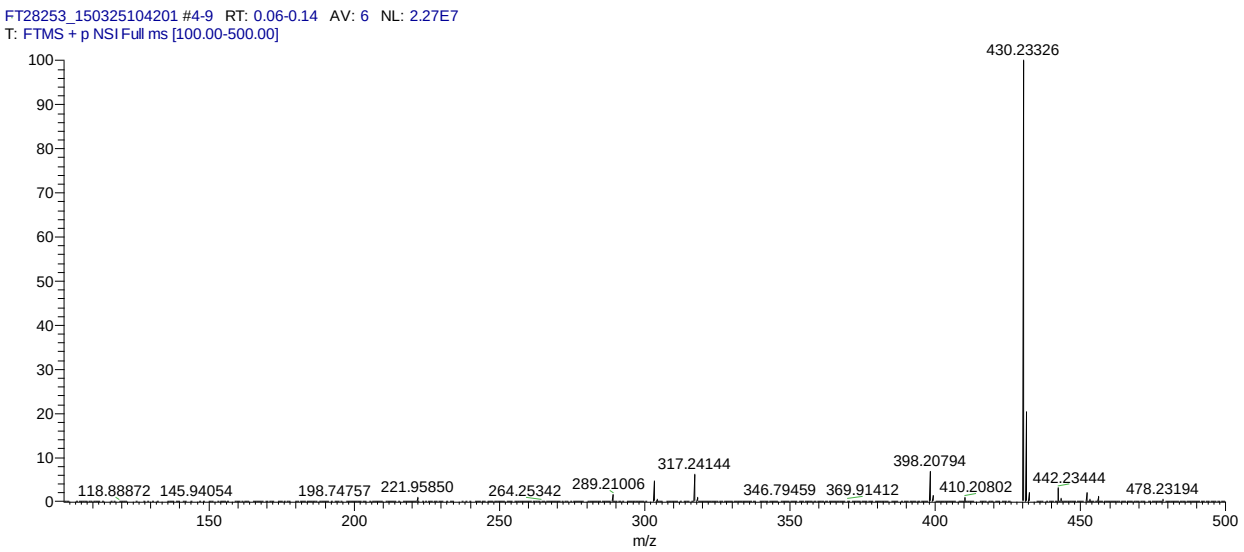


Figure 5. Mass spectrometry data of the SFF-acetal peptide. The single most prominent m/z peak is 430.23326, which is the mass of a singly-charged SFF-acetal peptide.

The same procedure outlined previously was used to prepare the hSFF-acetal sample for purification. This sample was purified using the new Waters 2998 HPLC system and the 30 by 250 mm XSelect C_{18} 10 µm prep column. The hSFF-acetal peptide was suspected to elute at a high acetonitrile concentration than the SFF-acetal, so a similar probing run was conducted from 25% to 65% acetonitrile in 40 minutes at a flow rate of 60 ml/min. Unfortunately, no significant peak was observed on the chromatograph. Therefore, the sample was concentrated and re-injected, and a small peak was observed to elute at 45% acetonitrile. For each injection, the fraction that eluted at 45% acetonitrile was collected, and all the collected fractions were combined, reduced to a volume of 20 ml in vacuo, and lyophilized. A small portion of the dried product was then dissolved in methanol and submitted for an exact mass spectrometry. However, the mass spec data indicated that the sample, unfortunately, wasn't the hSFF-acetal. This essentially meant that all synthesized homoserine peptides have been consumed and lost, and that a DCL of hSFF-aldehydes will have to be constructed and investigated at a later time.

Part 2: Construction and Characterization of the DCL

To restore the acetal group to an aldehyde, the peptide must be dissolved in an acidic solution, and the solution must be removed completely after 30 minutes. An aqueous 4% HCl solution was made using HPLC grade water. 4.02 mg of the SFF-acetal peptide was obtained and dissolved in 2.34 ml of the 4% HCl solution to make a 4 mM solution. As soon as the peptides were completely dissolved, the solution began drying in vacuo; within 20 to 25 minutes, the acidic solution was completely removed. The first DCL was established at 40% acetonitrile-60% water at pH 7. The dried and restored peptide aldehyde was dissolved again in 2.34 ml of the 40% acetonitrile solution. The pH of the mixture was then adjusted to exactly 7 using a 5% triethylamine solution and centrifuged using the Eppendorf Centrifuge 5810R at 14000 rpm and 4℃ for 25 minutes. The pelleted insoluble portions of the mixture were left untouched, and the soluble portions of the mixture, which constitutes the DCL, were collected. At this point, the DCL has been established, and the system was kept at 4℃. A DCL of 20% acetonitrile at pH 7 was also created using the same procedure.

At time points of 0 hrs, 2 hrs, 4 hrs, 24 hrs, 48 hrs, 96 hrs, and 7 days, a portion of the DCL was allocated for analysis in order to identify the chemical species that are present in the library. The system at each time point was analyzed using the Waters analytical HPLC system in the Lynn lab (996 autosampler, Delta 600 pump) on a 4.6 by 250 mm Atlantis 5μm T_3 column. The method used was 10% to 100% acetonitrile in 90 minutes at 1 ml/min. For the 0 hr and 2 hrs time point, 50 μl of the library was obtained, mixed with 50 μl of pure acetonitrile, and immediately inserted into the autosampler for HPLC analysis. For the 4 hrs sample and onwards, 75 μl of the library was obtained in order to enlarge the peak sizes on the HPLC chromatograph. The peaks were then integrated in order to measure the proportion of each chemical species

(monomers, dimers, etc) in the DCL (table 1a). This process was repeated for the 20% acetonitrile DCL (table 1b).

Table 1. The numbers below the monomer and dimer columns represent each specie's peak area as it elutes. The wavelength used for analysis was 258 nm, which is the characteristic wavelength for phenylalanine. The total area was calculated by adding the monomer and dimer areas. [Monomer] was calculated by its area divided by the total area times 4 mM. [Dimer] was calculated by its area divided by total area times 4 mM divided by 2 (since it's a dimer). The asterisk indicates a sample that was rapidly frozen then thawed the next day for analysis due to instrument availability issues. The double asterisk indicates the 20% acetonitrile DCL batch that was sonicated (suspicions that significant precipitation has sequestered much of the dimers) before it was analyzed via HPLC.

A).

Time (hr)	Monomer	Dimer	Total	[Monom.]	[Dimer]
0	918358	97708	1016066	3.62	0.19
2	826727	151361	978088	3.38	0.31
4	1047169	401637	1448806	2.89	0.55
24	557287	594894	1152181	1.93	1.03
50	438866	720620	1159486	1.51	1.24
96	375039	783570	1158609	1.29	1.35
168	311850	781626	1093476	1.14	1.43

B).

Time (hr)	Monomer	Dimer	Total	[Monom.]	[Dimer]
0	1022910	93540	1116450	3.66	0.17
2	781526	134918	916444	3.41	0.29
4	818492	183294	1001786	3.27	0.37
25	538974	272642	811616	2.66	0.67
48*	322845	301755	624600	2.07	0.97
96	448893	263955	712848	2.52	0.74
190	480291	172345	652636	2.94	0.53
192**	560513	300025	860538	2.61	0.70

Graphical representations of the growth of chemical species in both DCLs were produced using the data provided in the preceding tables (figures 6, 7a, and 7b). It appears that for the 40% acetonitrile DCL, the growth of the dimeric species proceeded rapidly until it tapered off around 1.5 mM. A MALDI conducted on the 40% DCL on day 14 indicated the existence of both the cyclic dimer (MW of 731g) and linear dimer (MW of 749g). It is predicted that the linear and the cyclic dimers would have different hydrophilicity and would appear as two different peaks as they elute from the HPLC. However, only two peaks (one for monomers and one for dimer) were

observed on the analytical HPLC chromatograph between 0 hour and 7 days. Since the acidity of the MALDI matrix is known to break N,O-acetal linkages (instead of forming new ones), the dimer is most likely cyclic, which adheres to the results from previous studies using STF-CHO.[11] Nevertheless, an LC/MS is required to separate the chemical species present in the DCL and to identify each as monomers, linear dimers, and/or cyclic dimers. No other chemical species such as trimers were observed in noticeable quantities. During the 7 days of the experiment, little but noticeable precipitation occurred; therefore, none of the batches were required to be sonicated in order to release the chemical species that are sequestered in the precipitation. For the 20% acetonitrile DCL, significant precipitation appeared by the 24 hour time point. However, the idea to sonicate the sample before injection did not occur until the last sample, taken at 8 days. In figure 7a, which uses the data point from an un-sonicated sample, the concentration of the dimer appears to be in decline. In figure 7b, which uses the data point from the sonicated sample, the concentration of the dimer appears to stabilize around 0.70 mM. This indicates that many dimers in the DCL have indeed been sequestered as they precipitated out of the solution. It also indicates significantly poorer solubility for the dimeric species in an environment with less acetonitrile.

For the graphs 7a and 7b, the dimeric concentration at 48 hours appears noticeably higher than the equilibrium concentration of 0.70 mM. This is because the 20% acetonitrile library was not analyzed immediate at 48 hours due to the HPLC's unavailability. Instead, two samples were collected at the 48th hour and were both rapidly frozen with liquid nitrogen. One sample was kept frozen, and the other was lyophilized overnight. The frozen sample was rapidly melted under running warm tap water and analyzed using the HPLC the next day. The lyophilized sample, however, did not fully re-dissolve. The data from the frozen sample was used as the 48 hour time point, which seems to indicate growth in dimer concentration in the first 48 hours

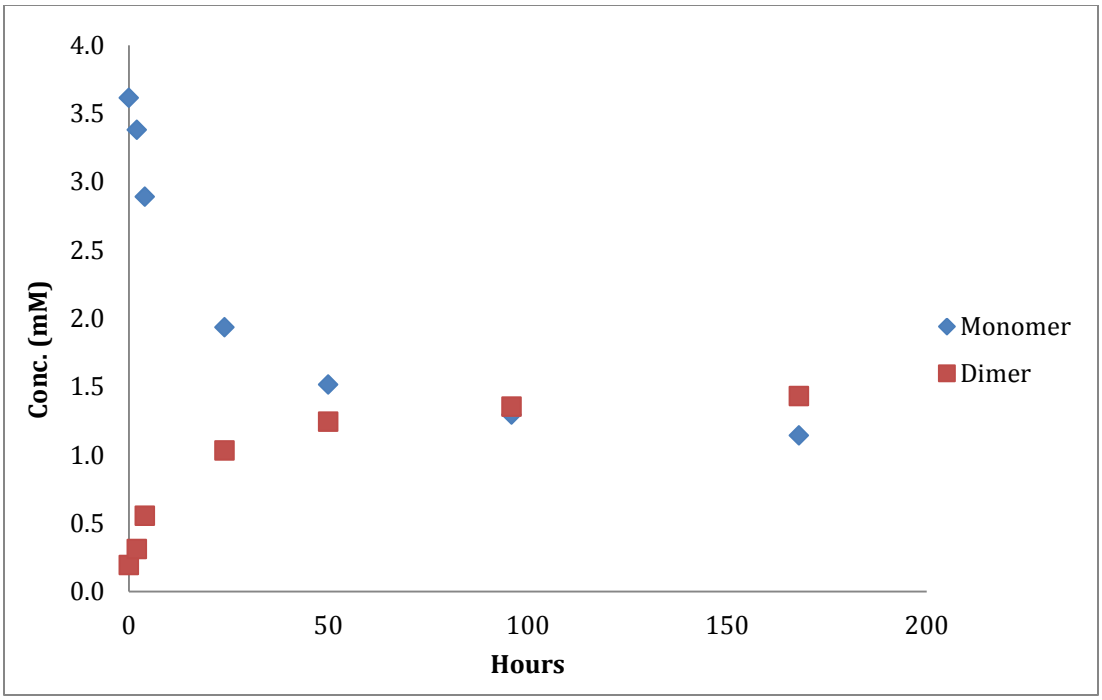
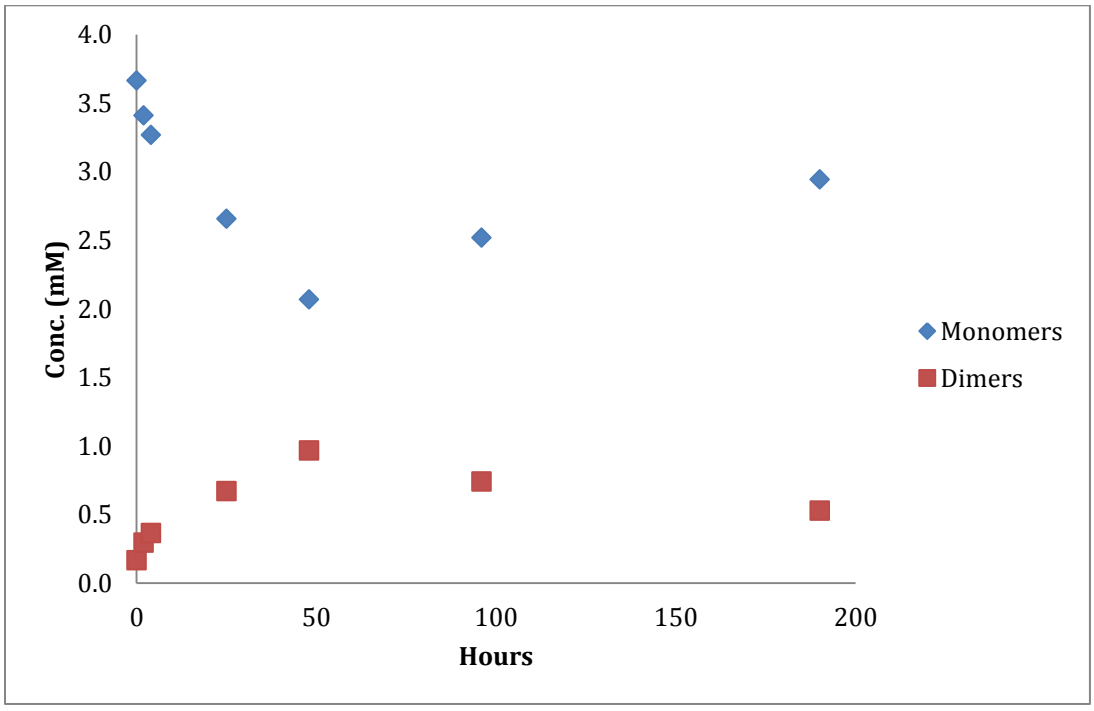


Figure 6. The graphical representation of the growth/decline in concentration of both observed chemical species for the 40% acetonitrile DCL.

A).



B).

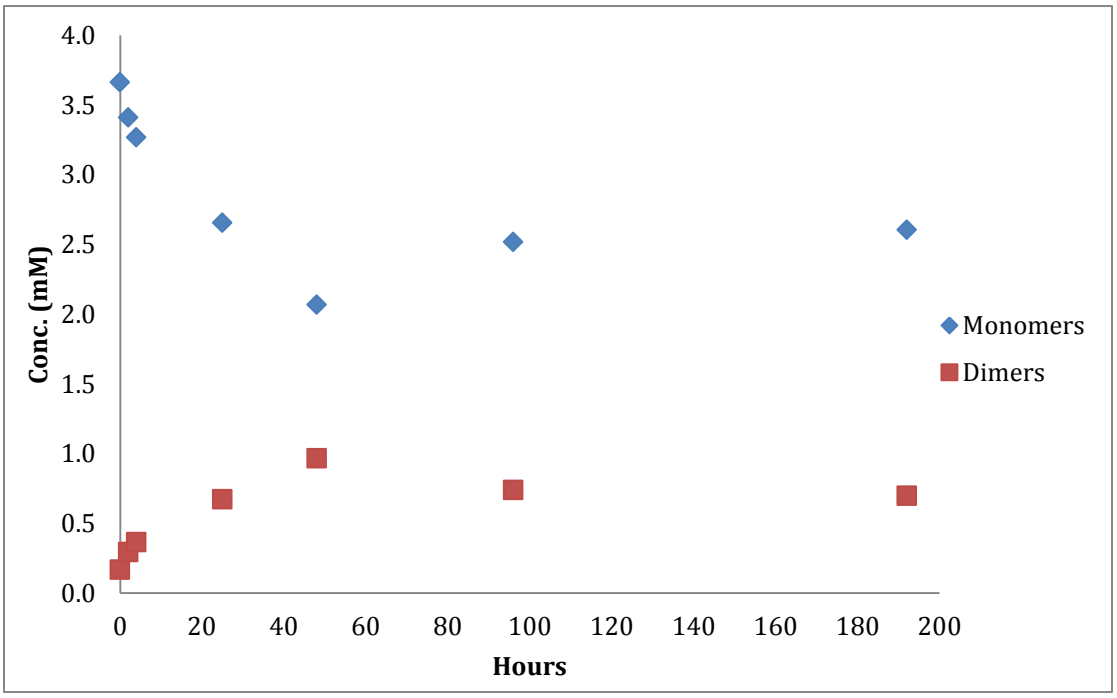


Figure 7. The graphical representation of the growth/decline in concentration of both observed chemical species in the 20% acetonitrile DCL. Figure 7a uses the 190th hour time point (8 days minus 2 hours), which was not sonicated prior to analysis. Figure 7b uses the 192th hour time point (exactly 8 days), which was sonicated prior to analysis.

followed by a decline. In the 20% acetonitrile DCL, no other chemical species were observed except for unreacted monomers and dimers. A control experiment was planned in which two samples would be allocated from the DCL; one sample would be analyzed on time, and the other would be immediately frozen and then analyzed the next day in order to ascertain the validity of a frozen sample. However, this experiment was unfortunately not conducted due to limited instrument availability; it will be conducted in the near future. In both DCLs, equilibrium seemed to have been reached by the 50th hour, although the composition of the dimer in the 40% acetonitrile DCL still seemed to increase by the 7 day time point. The period of rapid growth seems to be between 0 and 48 hours in both DCLs. Therefore, the control experiment should be conducted ideally between the 4 hour and the 24 hour time points.

Using the analytical HPLC method (10% to 100% acetonitrile in 90 minutes), the monomeric specie elutes around 24 minutes (34% acetonitrile), and the dimeric specie elutes around 47 minutes (57% acetonitrile). In forming the dimeric specie, an ionic N-terminus amine is consumed, and a dimer with four phenylalanines is produced. As a result, this new specie is even more hydrophobic and less soluble in the DCL environment compared to the monomeric specie. Likewise in the 20% acetonitrile DCL (in which the dimer is even less soluble than in the 40% acetonitrile DCL), the concentration of the dimeric specie is noticeably less than in the 40% acetonitrile DCL. Using each chemical species' solubility as a predictor of its formation, it appears even less likely that a linear trimer would be formed. This theory however, must be rigorously tested in the future on DCLs composed of other peptides with hydrophilic residues in order to support its validity.

The supramolecular structures present in the 40% acetonitrile DCL at 3 hrs, 24 hrs, 48 hrs, 96 hrs, and 7 days were also investigated. On each TEM grid (carbon film on 200 mesh copper), 6 μl of the DCL was dotted and allowed to stick for 2 minutes. It was also found that a film often formed on the surface of the dotted sample; therefore, an 8-fold diluted sample was also made. This diluted sample was then dotted onto a separate grid and also allowed to sit and stick for 2 minutes. After the 2 minutes, the sample was dried from the grid surface with a tissue and allowed to dry for around 1 minutes before being stained with a tungsten stain (20 mg/ml methylamine tungstate in water) for 2 minutes, at which point the stain was dried from the grid surface as well. For the 20% acetonitrile DCL, TEM samples were collected at 0 hr, 24 hrs, 48 hrs, 96 hrs, and 7 days, and the plating and staining procedures are the same as previously discussed. The 96 hr and 7 day samples for the 40% acetonitrile DCL and the 0 hr, 24 hrs, 48 hrs,

and 96 hrs samples for the 20% acetonitrile DCL were allowed to dry for a minute after plating and prior to staining; all other samples were stained immediately after plating.

For the 40% acetonitrile DCL faint outlines of what appears to be fibers are stemming out of clusters of peptide aggregates by the 3 hour time point. Likewise at the 24 hour time point, faint outlines of small fibrous structures were observed (figures 8a and 8b). At the 48 hour time point, these fibrous structures appeared to have grown in size (figure 9a). At the 96 hour (figure 9b) and 7 day time points, however, no fibrous structures, or any discernible structures, were found. Nevertheless, there appears to be significant amounts of peptide aggregates on the 7 day time point TEM sample (figures 10a and 10b). Although the progression of the TEM images indicate that the growing fibrous structures suddenly collapsed into aggregates, previous experiences dictate that this kind of occurrence is unlikely, and that more diverse structures were supposed to follow. One of the most likely explanations for this bizarre observation is that the peptides were poorly stained, which would require this experiment to be repeated. Another explanation could be that aggregates are indeed a thermodynamically more stable product than the fibrous structures. In order to provide more validity towards any of these conjectures, more experiments must be conducted.

For the 20% acetonitrile DCL, only the sample at 7 days was analyzed using TEM due to limited instrument availability (figures 11a and 11b). The dominant structure observed were fibers, similar in appearance to those observed for the 40% acetonitrile DCL at the 48 hour time point. In order to provide a more cohesive and definitive idea regarding the kinetics of structural development in the 20% acetonitrile DCL, the DCL must be analyzed at all the previous time points (0, 2, 4, 24, 48, and 96 hour). To provide clues to answer whether or not the aggregates are indeed more thermodynamically stable than the fibrous structures, additional samples must

be obtained for TEM imaging at later time points such as at 10 days, even 2 weeks. At this point, there's not enough data to make any more comparisons and to comment any further regarding structural development in these diphenylalanine-based peptide aldehyde DCLs.

Conclusion

In summary, this project involved two parts: synthesis and modification of the peptide based materials, and the establishment and study of the peptide-aldehyde dynamic combinatorial libraries (DCLs). The NMR and mass spectrometry data presented above shows proof that the peptides have been successfully made. However, the data gathered thus far on the SFF-aldehyde DCL does not allow much further comparisons to be made. It can be gleaned from this study, however, that the less hydrophilic species (dimers) were formed in less abundance in a less soluble environment, which suggests a possible link between solubility and the formation of additional chemical species such as trimers. Furthermore, TEM studies did reveal that the system is capable of self-assembly, although few supramolecular structures were observed for the 40% acetonitrile library beyond the 48 hour time point.

This study must be continued and repeated on the hSFF-aldehyde. One of the tricky aspects of synthesizing and chemically modifying peptides is that depending on the residues of the peptide, they are sometimes capable of self-catalysis due to their side chains. Several explanations have been proposed as to why the hSFF-aldehyde was lost. One explanation is that during the Fmoc removal process, the sidechain of the homoserine attacked the carbonyl group of the Fmoc protection group under the basic conditions and formed a six-member ring, thus removing an ionic N-terminal amine that may allow the peptide to be dissolved in water. Furthermore, Fmoc-hSFF-aldehyde and Fmoc-SFF-aldehyde, when examined using TLC, appears to have drastically different hydrophilicity; thus another explanation could be that during

the H_2O/ether washing and extraction process, hSFF-aldehyde was simply too hydrophobic to be extracted by water. Thus, it may have stayed dissolved in the ether layer and was discarded.

The SFF-aldehyde DCL must be also further analyzed as well. The kinetic studies should be extended to 10 days or even more to account for potential chemical species that may form more slowly. One of the downfalls of this study is the insolubility encountered during various stages of this study, from purification to forming and examining the DCLs. For example, due to the insolubility of the SFF-aldehyde in the 20% acetonitrile DCL, the sample has to be sonicated in order to account for all of the dimers that are formed, instead of just the ones that are not sequestered by the precipitation. In order to better visualize the supramolecular structures, a different stain could be used in future studies. Uranyl acetate is the stain most commonly used to stain samples similar to SFF-aldehyde; however, methylamine tungstate was used instead because uranyl acetate wasn't available. It is unlikely that fibrous networks that were beginning to form would completely disappear. Furthermore, fibrous structures were observed at 7 days in the 20% acetonitrile DCL, which lends further evidence to the hypothesis that the previous samples may have been poorly stained. What was observed in the 40% acetonitrile DCL does not correlate well with the diversity of supramolecular structures predicted by other studies. This study has also failed to achieve the trimeric species. If the solubility theory stated previously is indeed valid, then the next experiment (which incorporates the use of homoserine) should be conducted with threonine-phenylalanine peptides instead of diphenylalanine peptides. The threonine would ideally eliminate the insolubility problems.

Reference

1. Zhang, S., "Fabrication of novel biomaterials through molecular self-assembly," *Nature Biotechnology*, vol. 21, no. 10, Oct. 2003, pp. 1171-1178.

2. Djalali, R., Chen, Y., and H. Matsui, "Au Nanowire Fabrication from Sequenced Histidine-Rich Peptide," *JACS*, 124(46), Oct. 2002, pp. 13660-1366.

3. Matsui, H., Porrata, P., and G. E. Douberly, Jr., "Protein Tubule Immobilization on Self-Assembled Monolayers on Au Substrates," *Nano Letters*, 1(9), Aug. 2001, pp. 461-464.

4. Hartgerink, J., Beniash, E., and S. Stupp, "Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers," *Science*, 294, Nov. 2001, pp. 1684-1688.

5. Silva, G., Czeisler, C., Niece, K., Elia, B., Harrington, D., Kessler, J., and S. Stupp, "Selective Differentiation of Neural Progenitor Cells by High-Epitope Density Nanofibers," *Science*, 303, Feb. 2004, pp. 1352-1355.

6. Brewer, A., and A. Davis, "Chiral encoding may provide a simple solution to the origin of life," *Nature Chemistry*, 6, Jul. 2014, pp. 569-574.

7. Cairns-Smith, A. G. *Genetic Takeover and the Mineral Origin of Life* (Cambridge Univ. Press, 1982).

8. Cairns-Smith, A. G. Chemistry and the missing era of evolution. *Chem. Eur. J.* **14**, pp. 3830–3839 (2008).

9. Joyce, G.F., Visser, G.M., van Boeckel, C.A.A., van Boom, J.H., Orgel, E., and J. van Westrenen, "Chiral selection in poly(C)-directed synthesis of oligo(G)," *Nature*, 310, Aug. 1984, pp. 602 – 604.

10. Rubinov, B., Wagner, N., Rapaport, H., and G. Ashkenasy, "Self-Replicating Amphiphilic β-Sheet Peptides," *Angew. Che. Int. Ed.*, 48, 2009, pp. 6683-6686.

11. Nguyen, R., Allouche, L., Buhler, E., and N. Giuseppone, "Dynamic Combinatorial Evolution within Self-Replicating Supramolecular Assemblies," *Angew. Chem. Int. Ed.*, 48, 2009, 1093-1096.

12. Corbett, P. T., Leclaire, J., Vial, L., West, K., Wietor, J., Sanders, J., and S. Otto., "Dynamic Combinatorial Chemistry," *Chem. Rev.*, 2006, 106(9), pp 3652-3711

13. Frederix, P., Ulijn, R., Hunt, N., and T. Tuttle, "Virtual Screening for Dipeptide Aggregation: Toward Predictive Tools for Peptide Self-Assembly," *The Journal of Physical Chemistry Letters*, 2, 2011, pp. 2380-2384.

14. Liu, J., Zhang, E., Tan, J., and D. Lynn, (2014, April). "Exploring the Emergence of Biopolymers in Dynamic Chemical Networks," Poster presented at the Undergraduate Research Symposium at Emory University, Atlanta, GA.

15. Guo, C., Luo, Y., Zhou, R., and G. Wei, "Probing the Self-Assembly Mechanism of Diphenylalanine-based peptide nanovesicles and nanotubes," *ACS Nano*, Vol. 6, No. 5, 2012, pp. 3907-3918.

16. Yan, X. H., Zhu, P. L., and J. B. Li, "Self-Assembly and Application of Diphenylalanine-Based Nanostructures," *Chem. Soc. Rev.*, 39, 2010, pp. 1877-1890.

17. Adler-Abramovich, L., Reches, M., Sedman, V.L., Allen, S., Tendler, S. J. B., and E. Gazit, "Thermal and Chemical Stability of Diphenylalanine Peptide Nanotubes: implications for Nanotechnological Applications." *Langmuir*, 22, 2006, pp. 1313-1320.

18. Kim, J., Han, T. H., Kim, Y., Park, J. S., Choi, J., Churchill, D., Kim, S. O., and H. Ihee, "Role of Water in Directing Diphenylalanine Assembly into Nanotubes and Nanowires," *Adv. Materials*, 22(5), Sept. 2009, pp. 583-587.

19. Liu, J., Tan, J., and D. Lynn, (2014, August). "Exploring the Emergence of Biopolymers in Dynamic Chemical Networks," Poster presented at the Summer Undergraduate Research Experience Symposium at Emory University, Atlanta, GA.

A). B).

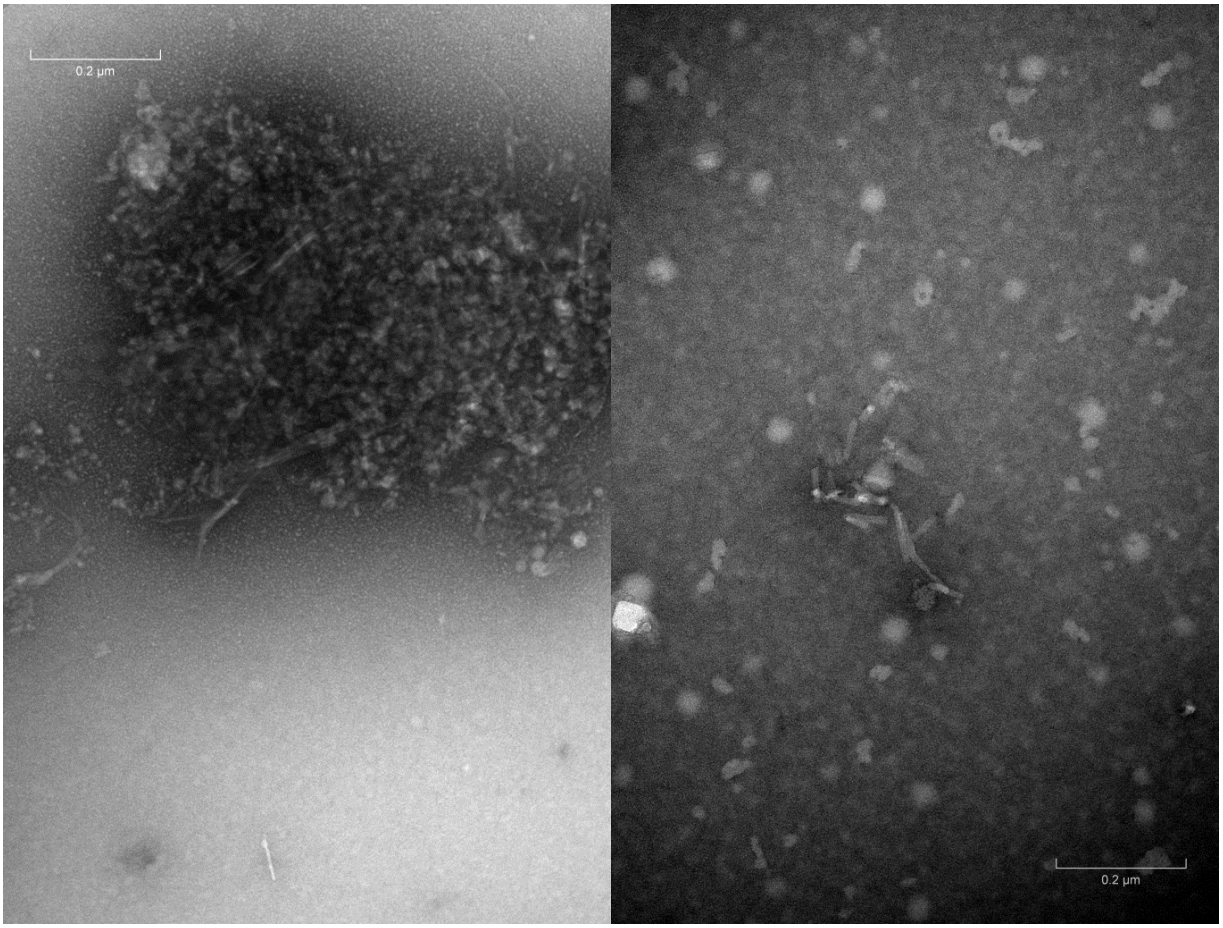


Figure 8. A) TEM image taken at 3 hours of what appears to be fibrous structures growing out of a cluster of peptide aggregates. B) TEM image taken at 24 hours shows faint outline of fibrous structures.

A). B).

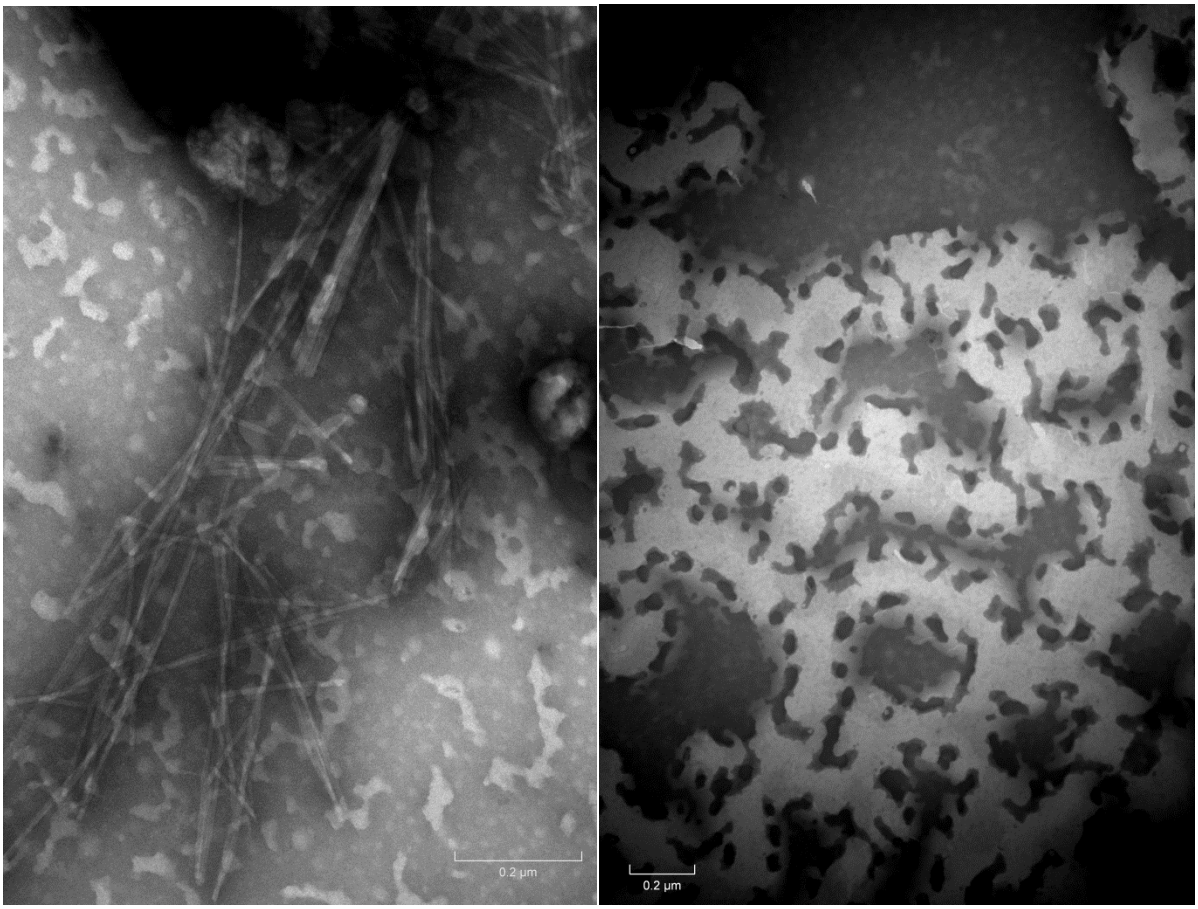


Figure 9. A) TEM image taken at 48 hours depict clear outlines of the growing fibrous structures. However, the TEM image taken at 96 hours (B) does not show any signs of aggregation or supramolecular structures.

A). B).

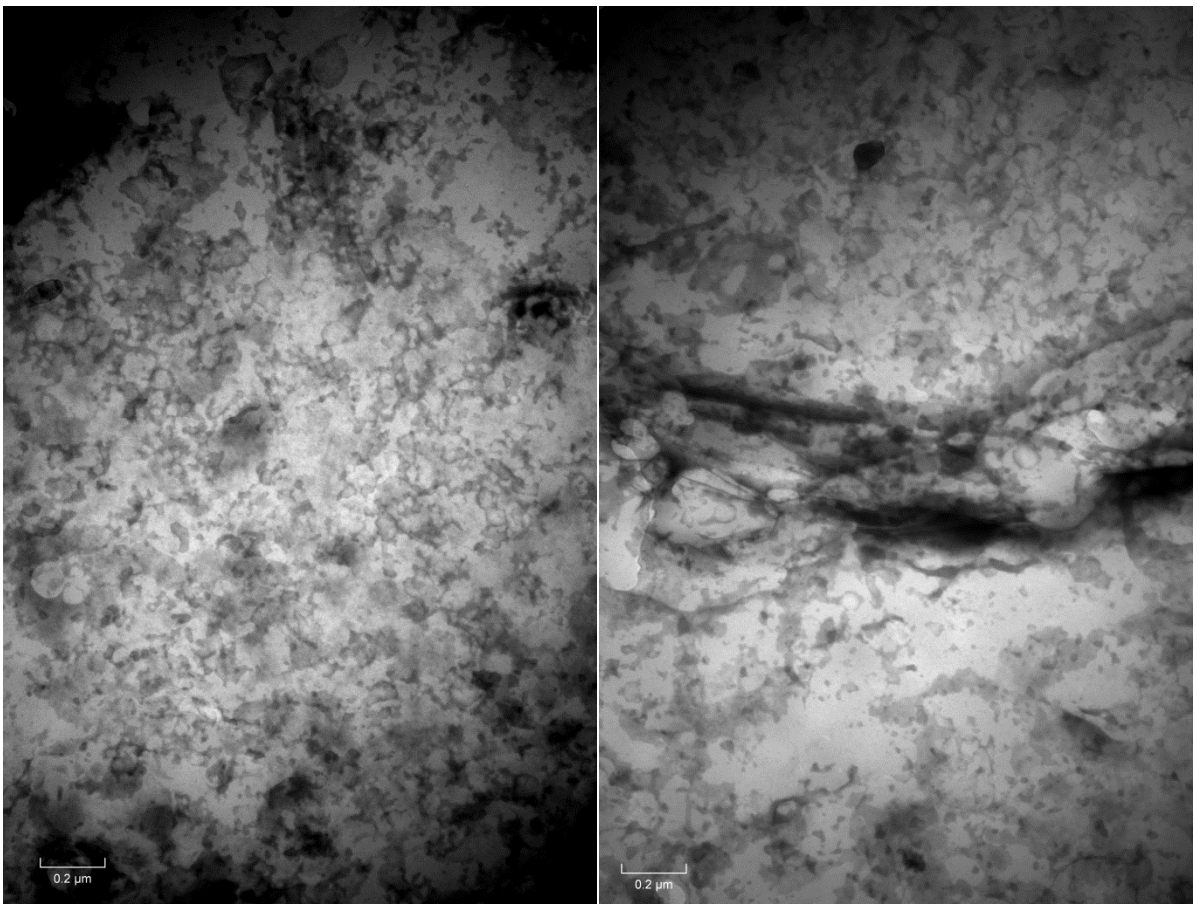


Figure 10. TEM images taken at 7 days. Both (a) and (b) depict peptide aggregates. However, no signs of supramolecular structure were seen.

A). B).

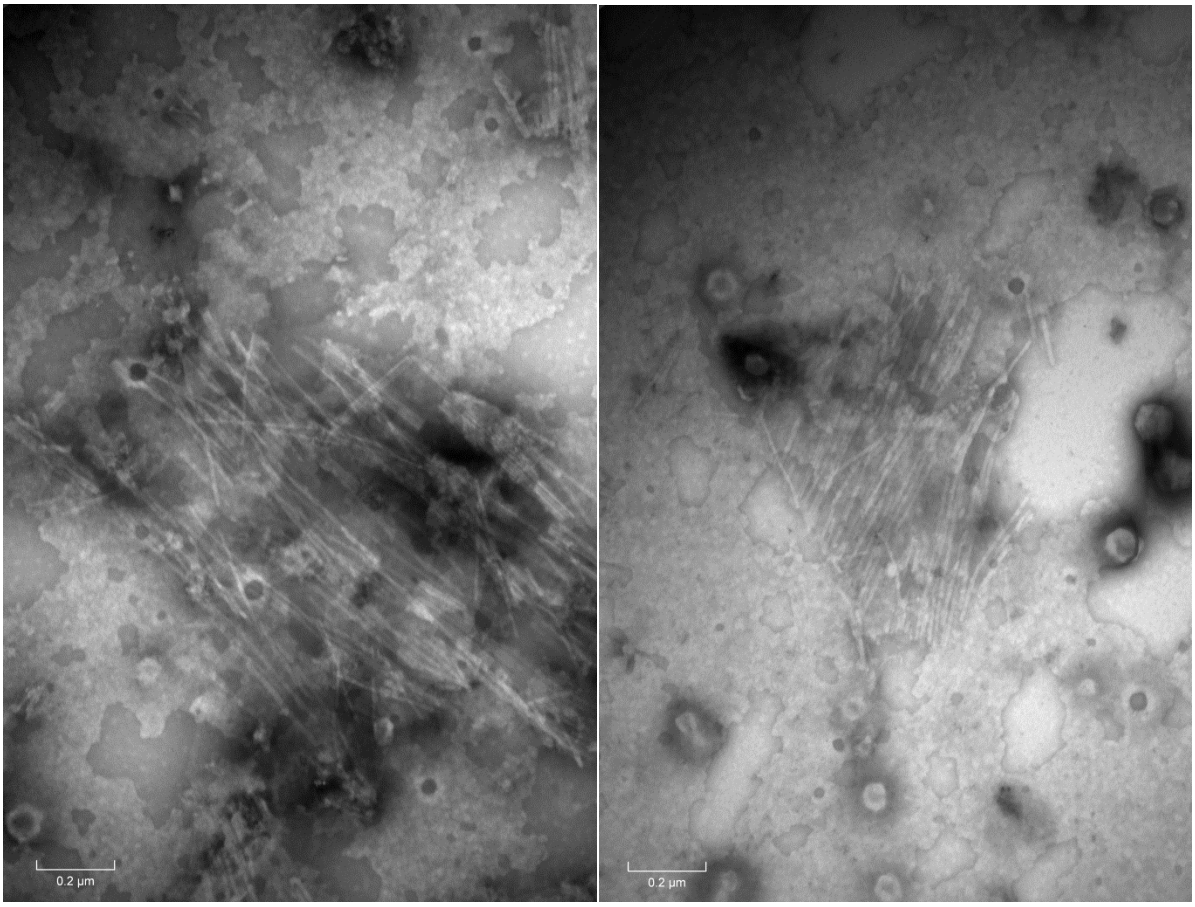
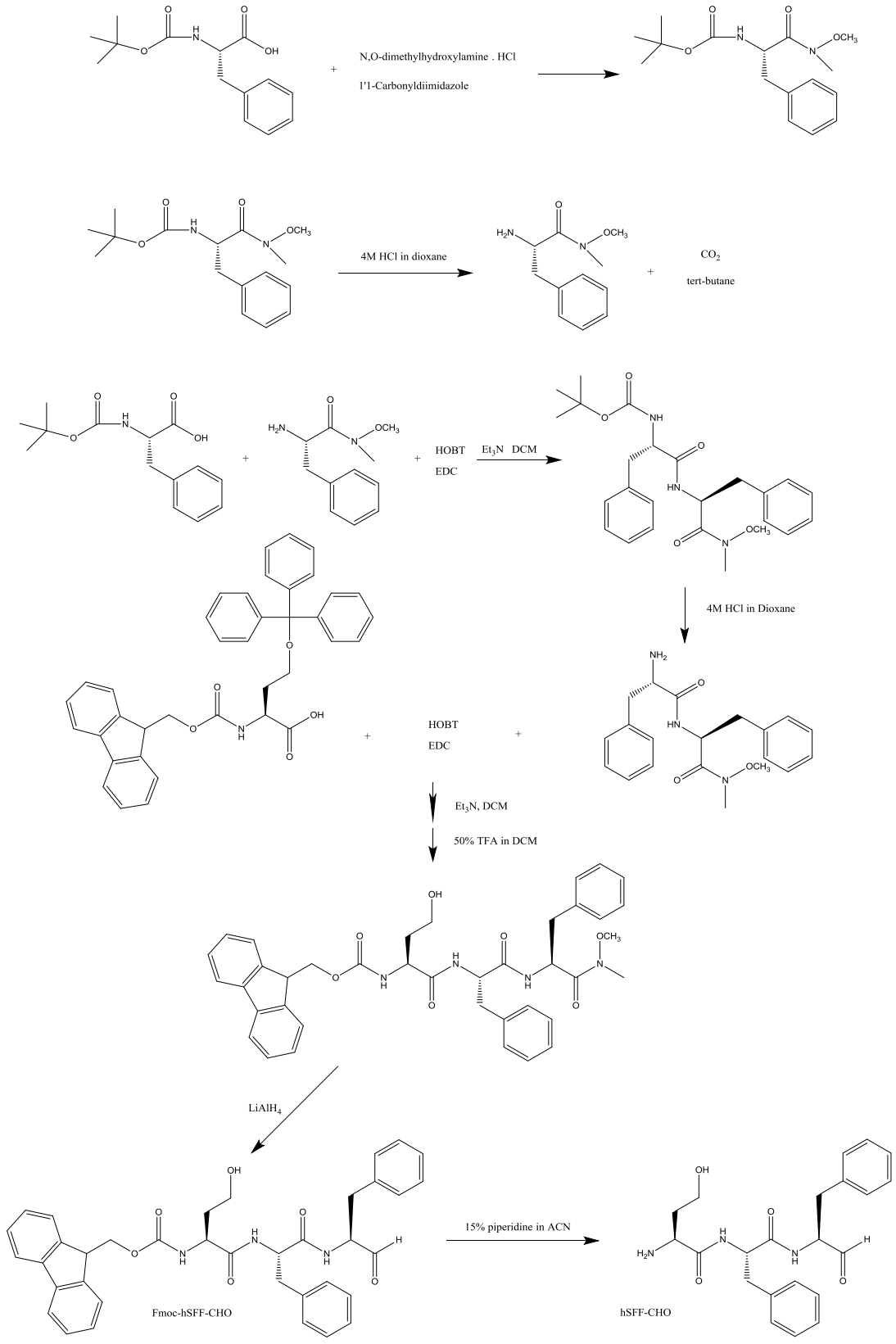


Figure 11. TEM images taken at day 7 of the 20% acetonitrile SFF-aldehyde DCL. Clear fibrous structures are outlined by the stain. These resemble the structures observed at 48 hours in the 40% acetonitrile SFF-aldehyde DCL.



Scheme 12. The synthetic roadmap of hSFF-aldehyde.

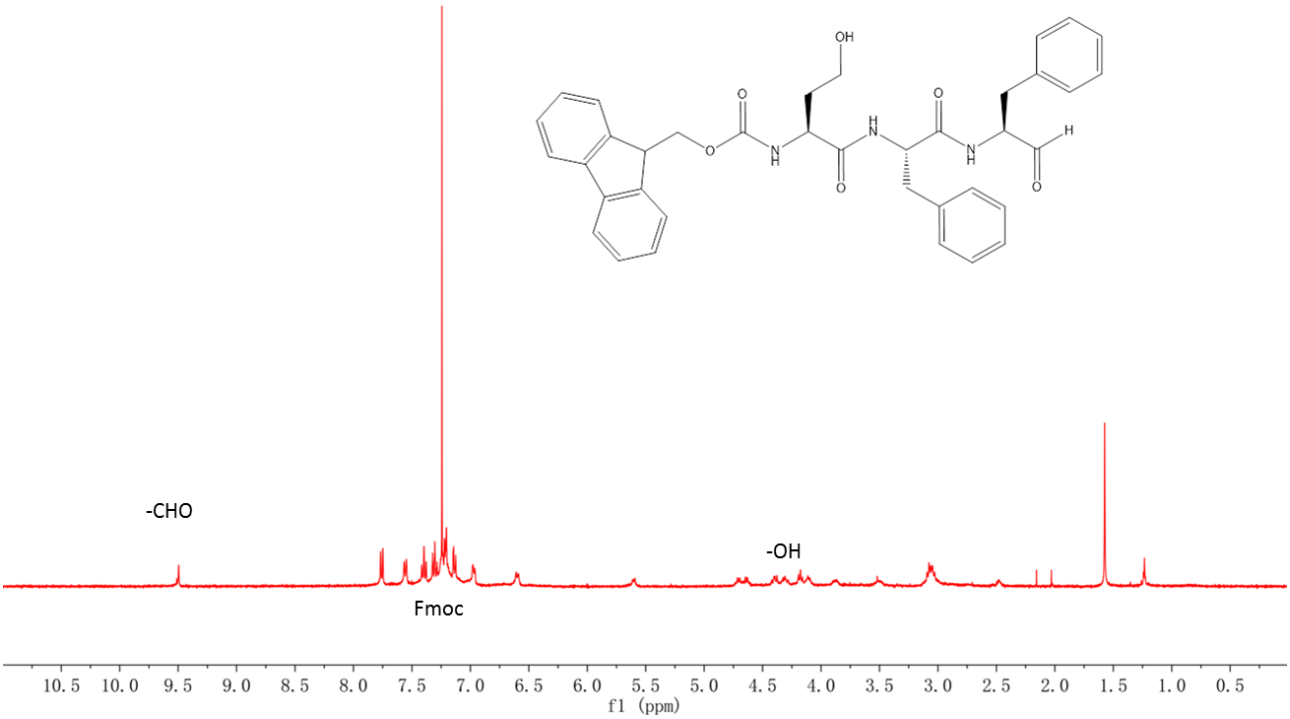


Figure S1. The NMR graph of the semi-purified hSFF-aldehyde. The appearance of the peak at 9.5 ppm, the Fmoc protons between 7 and 8 ppm, and the disappearance of two sharp peaks at 3.6 and 3.2 ppm indicate that the vast majority of the unreacted peptide-weinreb amide has been removed.