Genetically Engineered Peptides for Light Harvesting and Folding Kinetics Studies

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Genetically Engineered Peptides for Light Harvesting and Folding Kinetics Studies

An honors thesis presented to the
Department of Chemistry
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and graduation from the Honors College

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ABSTRACT

The preparation and characterization of β-sheet forming polypeptides for use in light harvesting applications is described. The Higashiya genetic cassette strategy (HGCS) was employed in order to make DNA libraries of repetitive DNA sequences. These DNA libraries contained gene cassettes coding for proteins designed to fold into the desired secondary motif. The conditions required for peptide expression, folding and formation of micron length fibrils was determined for the purpose of photophysical characterization, such as fluorescence lifetimes and acceptor dye quantum yields. Of special interest was the investigation of an “antenna effect” under FRET illumination, the intensity of acceptor dye emission was examined as a consequence of increasing peptide length. When β-sheet forming polypeptides bearing tryptophan residues along one edge were irradiated at 280 nm, the tryptophan absorbance maximum, the fluorescence intensity of the C-terminal acceptor chromophore, N-(7-dimethylamino-4-methyl) coumarin (DACM), increased proportionally with an increasing number of donor tryptophans. The fluorescence intensity increased even as the length of the β-sheet edge approached 256 Å, well beyond the Förster radius for the tryptophan-acceptor chromophore pair. Folding of the polypeptides was examined as well using Circular Dichroism, and fibrillation was examined using ThT kinetic studies. Beta sheet formation was detected via Circular Dichroism, and fibrillation was confirmed by the ThT kinetics studies. The plots of CPS vs. time showed an increase in florescence which was indicated by the positive slope of the plot, this positive slope shows that fibrillation is occurring.
ACKNOWLEDGEMENTS

To my advisor Professor John T. Welch, my mentor Jason Seeley, and of course my loving friends and family, I want to sincerely thank you for the support and encouragement. Without you I would not be writing this thesis and I would not have gotten this far without you by my side.

I would like to also thank the Welch lab group for welcoming me and for their support and encouragement as well.

I would especially like to thank my dad for his faith in me and his encouragement to always push boundaries and think critically. Without you I would not be the scientist I am today.
TABLE OF CONTENTS

Acknowledgements ...........................................................................................................iii

Abstract ..........................................................................................................................ii

Table of contents ..........................................................................................................iv

Introduction ....................................................................................................................5-8

Materials and Methods .................................................................................................9-16

Results ...........................................................................................................................17-24

Discussion and Conclusions .........................................................................................25-26

Future Directions .........................................................................................................27-28

References ....................................................................................................................29
Introduction:

The HGCS provides a rapid route for the preparation of a series of genetically engineered polypeptides that assume β-sheet conformation. These ordered structures display confined tryptophan residues along the newly formed β-sheet edge with angstrom resolution, yielding electronically coupled supramolecular systems for investigation of the energy transfer dynamics. The self-assembled mature protein fibrils have π stacked tryptophan chromophores that serve as interconnected optical antennas. The chromophores are capable of extended energy transfer by way of exciton migration. Efficient energy transfer from π-π interactions resulting in molecular orbital overlap is central to this research. The tryptophan residues that serve as donor chromophores transfer energy to a covalently attached fluorophore. Resonance energy transfer between individual donor chromophores can increase the excited state population of the acceptor thereby amplifying the intensity of emitted light, through the phenomenon known as optical antenna effect. The unique physical properties of mature fibrillar peptides suggests utility in photovoltaic or other long range (multimicron) energy transfer applications. The genetically derived constructs may also be incorporated into plasmids that can direct in situ expression of small synthetic, photo-responsive peptides in cellular systems. These self-assembled polypeptides may also have applications in medicine since they can be easily incorporated into soft tissue, due to their innocuous nature.
Light harvesting is the study of molecules that capture photons such as organic dyes and pigments. Examples of light harvesting materials are chlorophyll, solar cells, organic photovoltaics, polymeric nanoantennas, Tobacco Mosaic Virus (TMV) coat protein, and DNA. Light harvesting is important to life processes such as photosynthesis in autotrophs where a light harvesting complex converts solar energy into chemical energy. Solar energy is first absorbed by pigments known as chlorophylls. These pigments are covalently linked to proteins that provide pigment molecules with the correct orientation and position. After light energy absorption by the pigment, energy is transferred to chlorophylls bonded to the respectively covalently linked proteins. The pigments and protein involved with this actual primary electron transfer event together form the reaction center. A large number of pigment molecules (500-1000) are collectively referred to as antenna, the pigment molecules act together to harvest light and transfer the light energy to the same reaction center. The purpose of the antenna is to maintain a high rate of electron transfer in the reaction center, even at lower intensities of light. The genetically engineered polypeptides also act as an antenna which can be compared to chlorophyll pigment molecules that act collectively as an antenna in a light harvesting complex. Tryptophan donor
residues in the peptide localize energy and serve together as optical antenna. The optical antennas localize energy received upon radiation and transfer that energy to an acceptor coumarin dye or fluorophore. Soft materials such as organogels and dendrimers have previously demonstrated light-harvesting functions. Peptide scaffolds offer an alternative mechanism to drive the self-assembly of chromophores.

Figure 2: The Light Harvesting Properties of Tryptophan in KWEWx Polypeptides

Donor Chromophore: Tryptophan serves as an optical antenna

model peptide KWEW4C1 GH [(GA)3 GK(GA)3 GW(GA)3 GE(GA)3 GW4 GAC] is labeled with N-(7-dimethyl amino-4-methylcoumarin-3-yl)maleimide (DACM), where tryptophan transfers energy to DACM
The KWEWx genetically engineered polypeptides could be used to target disease if they are functionalized with an antibody. Due to their light harvesting properties the polypeptides can be monitored fluorescently once the antibody binds to a diseased cell. The polypeptides can also have applications in material sciences (e.g. nanowires, nanotubes, optoelectronics, and quantum computers).

The polypeptides fold to assume γ turn beta sheets, and this has been determined using circular dichroism (CD) and Raman spectroscopy. It has been hypothesized that the type of aggregation that the polypeptides result in is fibrillation. The polypeptides would not be as stable if amorphous aggregates formed. Amyloid-like fibrils are found to be stable in a wide range of physiochemical environments that are not ordinarily tolerated by protein-based structures. The stability and morphological regularity of amyloid-like fibrils made these peptides an attractive starting point for the development of peptide-based self-assembling bio-nanomaterials. Amyloid fibrils are formed by normally soluble proteins that assemble insoluble fibers that are resistant to degradation. Fibril formation can accompany disease where each disease is characterized by the aggregation of designated proteins or peptides. Well known examples of amyloid diseases include Alzheimer's disease, Diabetes type 2 and the spongiform encephalopathies (e.g., Mad Cow Disease). The fibrillar assemblies are intrinsically stable, structural studies have revealed these assemblies are composed predominantly of β-sheet structure in a characteristic cross-β conformation.
Materials and Methods:

The polypeptide constructs are made using recombinant DNA technology. Initially KWEX gene cassettes are inserted into a high copy plasmid pUC18. A single DNA coding sequence expressing the monomer KWEX4 was cloned into the high copy plasmid puc18. The cloning reaction is typically 20 µL: 2 µL of pUC18 plasmid, 15 µL of the KWEX4 monomer with the adapter DNA sequence, 2 µL of buffer, this solution is then put in a water bath (42 °C) for one minute, 1 µL of DNA ligase is then added. The solution is gently mixed and left to incubate at room temperature for 2-4 hours. Then 1-2 µL of the cloning reaction is added to ~150 µL of XL1-blue cells that are on ice. The XL1-blue cells are used for DNA maintenance and propagation. The cells with the added cloning reaction are left on ice for one hour, after the one hour incubation period the transformed cells are heat shocked (placed in the 42 °C water bath for one minute and then put on ice for two minutes). 250 µL Super Optimal broth with Catabolite repression (SOC) is added to the transformed cells, the cells with the SOC media are then placed in the shaker at 37 °C for one hour. After the one hour incubation period in the shaker is done the cells are plated onto an LB-agar ampicillin plate.

The plate is then placed in an incubator (37 °C) for approximately one day. The cells that grow on the plate are screened for the desired gene cassette using colony PCR method, the cells with the desired gene are inoculated into ~5 mL of LB ampicillin media and put in the shaker overnight. The 5 mL culture is then introduced to a larger volume (500 mL) and left in the shaker until it reaches an optical density of 0.8 ODE. Once the culture has reached 0.8 ODE, it is pelleted down.
The pellets are placed in a freezer until needed for plasmid purification which is done using the Qiagen miniprep method. Once the pUC18-KWEW4 plasmid is purified the KWEW4 monomer is excised using the Type II restriction enzyme, BsaI. With a 20 µL reaction the amount of plasmid needed for the reaction is concentration dependent (based of the concentration after plasmid purification). The digest is then electrophoresed through an agarose gel containing ethidium bromide.

Figure 3: KWEW4 Digest with BsaI

Lane one has a 100 bp DNA molecular weight ladder, lanes 2-5 contain the KWEW4 monomer with a molecular weight of 400 bp. The circular and coiled DNA of the rest of the plasmid is found in the gel around 1000 bp.

The KWEW4 monomer is excised from the gel and the DNA is extracted and purified from the agarose gel using the Qiagen gel extraction method. An oligomerization can be affected using the purified KWEW4 monomer. The oligomerization is 20 µL: 17 µL of KWEW4 monomer, 2 µL 10x ligase buffer, the solution is placed in a 42 °C water bath for one minute, and then 1 µL of DNA ligase is added to the mixture. The oligomerization reaction is gently vortexed and centrifuged briefly in a tabletop centrifuge (~5000 rpm). The oligomerization reaction is most
effective if it is allowed to incubate at room temperature for a 12 hour period. The oligomerization is electrophoresed on an agarose gel containing ethidium bromide. The KWEW4 monomer has sticky ends that were created during the BsaI digest that will hybridize with KWEW4 monomer sticky ends (Figure 1). The phosphodiester backbone is sealed by DNA ligase. The multimers of KWEW4 are then fully formed.

Figure 4: Oligomerization of KWEW4 monomer

In lane one of Figure 4 is the 100 bp ladder, lane 2 contains the oligomerization reaction. Lane two illustrates KWEW4 monomer (at 400 bp) is present in the reaction mixture. There are oligomers in the gel around 800 bp (KWEW8) and 1200 bp (KWEW12). The concentration of the KWEW12 multimer is low as indicated by the low intensity of the band around 1200 bp.

The multimers (higher than 400 bp) are excised from the gel separately from the KWEW4 monomer and are purified using the Qiagen gel extraction method. The purified multimers are then further oligomerized with DNA adapter sequences. The adaptive DNA sequence is needed to insert the gene cassette into the cloning vector. The multimers with the adapter sequences were then electrophoresed and excised from the gel and once again purified using the Qiagen gel extraction method. The purified DNA repeats coding for the KWEW4 monomer as well as other
multimers were then cloned into the expression vector pET28. The cloning reaction required 20 µL: 17 µL of the monomer or multimer gene cassette with adapters, 1 µL of pET28 expression vector, and 2 µL of 10x ligase buffer, the mixture was then put in a 42°C water bath for one minute, and 1 µL of DNA ligase was then added to the mixture. The cloning reaction was then allowed to incubate for 2-4 hours. The purified expression constructs (pET28-KWEW4, pET28-KWEW8, pET28-KWEW12, pET28-KWEW16) were transformed into BLR(DE3)pLysS E.coli.

The transformed cells were seeded in a culture containing the appropriate antibiotic. Kanamycin and chloramphenicol were needed for the cells containing the KWEW4, KWEW8, and KWEW16 gene cassettes; the pET28-KWEW12 vector only carries a Kanamycin resistance gene therefore the cells containing this expression vector were only seeded in culture containing Kanamycin. The culture that was used for expression instead of LB media was 2×YT media which contains 16 g tryptone, 10 g of yeast extract, and 5 g NaCl per one liter of culture (pH = 7). The respective antibiotic added to each culture was 50 µg/mL. The cultures were induced by IPTG at a final concentration of 1 mM. All polypeptides expressed to date have been successfully purified under denaturing conditions (8 M urea) taking advantage of Ni-NTA technology. The expression cells were harvested 6 hours after the culture was induced with IPTG, the culture was centrifuged for thirty minutes at 4,000 rpm and 4 °C. Once the cells are harvested the cells are lysed with approximately 50 mL of 8 M urea. The lysed cells are centrifuged in Beckman centrifuge tubes in a 25.5 rotor at 20,000 rpm and 4 °C for one hour. The supernatant is the poured onto a Ni-NTA column and mixed for one day via rotation. The supernatant is then passed through the column, the column was washed with two 20 mL fractions of 8 M urea, the column was rinsed with two 10 mL fractions of 8 M urea with 20 mM imidazole. The peptide is then eluted in 6-8 5 mL elution fractions using 8 M urea with 300 mM imidazole. SDS-PAGE is was used to confirm purification
of the desired peptide. Plasmid sequencing of pUC18 and pET28 constructs containing the repetitive gene cassettes were successful, yielding 100% homology.

Figure 4: SDS PAGE Analysis of KWEW4

In lane one of Figure 4 is the Biorad Precision Plus Protein™ All Blue Protein Standards, lanes 2 through 7 contain the elution fractions. Lane two contains elution six, lane three contains elution five, lane four contains elution four, lane five contains elution three, lane six contains elution two, and lane 7 contains elution one. Lane 8 contains the second rinse fraction and lane 9 contains the first rinse fraction. Lane 10 contains the second wash fraction, lane 11 contains the first wash fraction, and lane 12 contains the flow through. All of the elution fractions contain a high concentration of KWEW4 peptide the concentration gradually decreases with each elution fraction, this correlates to a decrease in intensity of the band from lane 7 to lane 2. The broad band indicating KWEW4 in lanes 2-7 is right above 10 kDa.
Figure 5: SDS PAGE Analysis of KWEW8

In lane one of Figure 5 is the flow through, lane two contains the first wash fraction, and lane three contains the second wash fraction. Lane 4 contains the first rinse fraction, and lane 5 contains the second rinse fraction. Lanes 6-14 contain the elution fractions. Lane 6 contains the first elution fraction, lane 7 contains the second elution fraction, lane 8 contains the third elution fraction, lane 9 contains the fourth elution fraction, lane 10 contains the fifth elution fraction, lane 11 contains the sixth elution fraction, lane 12 contains the seventh elution fraction, lane 13 contains the eighth elution fraction, and lane 14 contains the ninth elution fraction. Lane 15 contains the SigmaMarker™ wide range marker. Elution two (lane 7) and elution three (lane 8) contain the most concentrated KWEW8 peptide, elution three contains the most peptide since the broad band right under 25 kDa has a higher intensity than the band in lane 7. The molecular weight of KWEW8 is approximately 23.5 kDa.

Figure 6: SDS PAGE Analysis of KWEW12

In lane one of Figure 6 is the Biorad Precision Plus Protein™ All Blue Protein Standards. Lane two contains the flow through, lane three contains the first wash fraction, and lane 4 contains the second wash fraction. Lane five contains the first rinse fraction, and lane six contains the second rinse fraction. Lane 7-12 contains elution fractions one through six. Lane 13 contains an elution fraction containing KWEW8 to have as a reference. All of the elution fractions contain concentrated KWEW12 indicated by the broad intense bands in lanes 7-12 under 37 kDa. The molecular weight of KWEW12 is approximately 30,890 g/mo.
In lane one of Figure 7 is SigmaMarker™ wide range marker, lanes 2 through 8 contain the elution fractions. Lane two contains elution seven, lane three contains elution six, lane four contains elution five, lane five contains elution four, lane six contains elution three, lane 7 contains elution two, and lane 8 contains elution one. Lane 9 contains the second rinse fraction and lane 10 contains the first rinse fraction. Lane 11 contains the second wash fraction, lane 12 contains the first wash fraction, and lane 13 contains the flow through. The first three elution fractions contain concentrated KWEW8 peptide (lanes 8-6). Elution three contains the most concentrated KWEW16 since it has a greater intensity than the bands in lane 7 and lane 8. The bands in lanes 6-8 indicating KWEW16 are above 45 kDa according to the molecular weight ladder.

Once the polypeptides are purified the elution fractions containing the concentrated peptide must be carbamylated in a boiling water bath for approximately two hours. When the polypeptides are heated in 8 M urea the lysine (K) residues are carbamylated to remove charge likely preventing electrostatic interactions between the peptides in solution. After the concentrated elution fractions (determined by SDS PAGE) have been carbamylated, dialysis must be set up in order to remove the 8 M urea. The concentration of the remaining urea should be in the nanomolar range. The carbamylated and concentrated elution fractions are placed in to dialysis tubing that is clamped with plastic clips at both ends. The dialysis tube is then placed in a liter of water. The number of times the dialysate must be changed can be calculated from the volume of the peptide elution fractions in the dialysis bag, the concentration of the urea (starting at 8 M), and the dialysate volume. On completion the peptide and water with nanomolar trace of urea was transferred to a conical vial. The conical vial can then be flash frozen with liquid nitrogen. The flash frozen sample can then be lyophilized to remove the water. The lyophilized sample can be re-solubilized in
sodium acetate buffer or phosphate buffer for folding and kinetics studies. The sample can also be dissolved in 8 M urea, Tris-HCl for DACM labeling.

Polypeptides used in resonance energy transfer (RET) experiments require labeling with an acceptable tryptophan FRET acceptor. The absorption and emission spectra of coumarin derivatives show that coumarin dyes are suitable FRET acceptors for tryptophan. Coumarin derivatives have high extinction coefficients, strong emission profiles, and sufficient spectral overlap for a successful FRET experiment. The Förster radius for tryptophan-DACM FRET pair is 31 Å. Lyophilized polypeptide samples were reconstituted in labeling buffer (8 M urea, Tris-HCl pH 7.2) and degassed under benchtop vacuum for one hour. Dithiolthreitol (DTT) was added at a specific concentration in order to reduce cysteine residues and prevent disulfide bond formation. After one hour incubation in DTT the polypeptide sample was added to Ni-NTA resin and gently mixed via rotation for at least two hours in order to promote protein binding. Residual DTT was removed to avoid interference with peptide-dye conjugation as DTT readily reacts with maleimides. A solution of dimethylamino coumarin in DMSO was then added to the denatured polypeptide at a 10-fold molar excess and then placed in a cold room for overnight storage with gentle rocking. After the overnight incubation period for protein binding, residual DACM was removed by washing the column with wash buffer. Due to the highly sensitive nature of fluorescence spectroscopy, removal of free DACM to limit background fluorescence is essential. Labeled polypeptides were eluted using six 5 mL portions of elution buffer (8 M urea, 300 mM imidazole). Elution fractions containing the labeled polypeptide were combined and extensively dialyzed against ddH$_2$O to remove imidazole and unreacted coumarin dye. Dialysis of the peptide-dye conjugates resulted in high levels of aggregation making isolation of the labeled polypeptides convenient by centrifugation and lyophilization.
Results:

Figure 8: Time Dependent CD Spectroscopy of Fluorescently Labeled KWEW8

In the circular dichroism spectra, a negative to positive transition at 197 nm, was observed consistent with the progression of protein folding. The circular dichroism confirms β-sheet structure.
The Raman spectrum exhibits an intense amide I band, indicative of β-sheet secondary structure. Additional β-sheet signature peaks observed include amide II, CαH, and amide III.

<table>
<thead>
<tr>
<th>Raman shifts</th>
<th>1200</th>
<th>1300</th>
<th>1400</th>
<th>1500</th>
<th>1600</th>
<th>1700</th>
<th>1800</th>
</tr>
</thead>
<tbody>
<tr>
<td>amide II</td>
<td>significant C-N str., N-H ben., C-C str.</td>
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<tr>
<td>amide III</td>
<td>significant C-N str., N-H ben., C-C str.</td>
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<tr>
<td>Cα-H</td>
<td>Cα-H sym ben., C-Cα str.</td>
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A fluorescence resonance energy transfer (FRET) experiment was conducted, and the absorption and emission spectra for donor tryptophan and acceptor \( N-(7\text{-dimethylamino}-4\text{-methycoumarin-3-yl}) \) were obtained.

Figure 11: FRET Efficiency as a Function of D-A Distance

A plot of FRET efficiency as a function of distance as shown in Fig. 9. The sigmoidal plot illustrates a 50 percent decrease in FRET efficiency when the distance between the donor and acceptor is just beyond 50 Å.
In FRET experiments, an increase in DACM fluorescence intensity correlated with increasing donor chromophore numbers that results with increasing peptide length. This provides evidence for the antenna effect. With increasing number of donors, DACM fluorescence intensity approaches that of the directly excited coumarin dye.

Figure 13: A Comparison of the Size of DACM labeled KWEWx Peptides
Figure 14: The Relative Fluorescence Intensity of Labeled KWEW8 at $\lambda_{280}$ and $\lambda_{373}$

Relative fluorescence intensity at 460 nm of labeled peptides KWEW8 C1-DACM irradiated at 280 nm (tryptophan donor absorption) and 373 nm (DACM absorption).

Figure 15: The Relative Fluorescence Intensity of Labeled KWEW16 at $\lambda_{280}$ and $\lambda_{373}$

Relative fluorescence intensity at 460 nm of labeled peptides KWEW16 C1-DACM irradiated at 280 nm (tryptophan donor absorption) and 373 nm (DACM absorption).
Figure 17: The Average Fluorescence Emission of KWEW4 (2.5 µM A,B,C)

Figure 18: The Average Fluorescence Emission of KWEW4 (5 µM A,B,C)
Figure 19: The Average Fluorescence Emission of KWEW4 (10 µM A,B,C)

Figure 20: The Average Fluorescence Emission of KWEW8 (2.5 µM A,B,C)
Figure 21: The Average Fluorescence Emission of KWEW8 (5 µM A,B,C)

Figure 22: The Average Fluorescence Emission of KWEW8 (10 µM A,B,C)
Discussion and Conclusions:

The positive ellipticity at 197 nm in Figure 8 clearly illustrated beta sheet structure of the KWEW8 labeled with DACM on Day 22 of the folding studies. From Day 1 through Day 16 of the folding studies the labeled KWEW8 peptide was in the random coil state indicated by the negative ellipticity at 197 nm. Beta sheet formation by KWEW8 labeled with DACM was further confirmed by Raman spectroscopy, the Raman spectra (Figure 9) has an intense amide I band which is diagnostic for beta sheet formation. Figure 10 shows energy transfer at which the tryptophan emission spectra overlaps or intersects the absorption spectra of the acceptor N-(7-dimethylamino-4-methycoumarin-3-yl) dye. This point of intersection of the spectra where energy transfer occurs is approximately 400 nm.

Figure 12 clearly displays the proportional increase in acceptor fluorophore fluorescence intensity with increasing donor chromophore residues associated with increasing peptide length. This antenna effect requires resonance energy transfer over lengths greater than the Förster radius. The tryptophan residues serve as optical antenna and localize energy. Increasing number of tryptophan residues lead to an increase in energy transfer from the donor chromophores to the DACM acceptor fluorophore. Due to the increase of D-A energy transfer with an increase in the amount of donor chromophores. Tryptophan emission decreases as the size of the peptide increases, and the fluorescence emission of the DACM dye increases (inversely related to tryptophan emission) as the size of the peptide increases. Figure 14 and Figure 15 show the fluorescence of labeled KWEW8 and KWEW16 respectively, when the peptides are irradiated at tryptophan donor absorption (λ<sub>280</sub>) and DACM (λ<sub>373</sub>) absorption. Both figures show an increase in fluorescence from irradiation at 280 nm to irradiation at 373 nm. The fluorescence intensity of KWEW16-DACM is higher than the fluorescence intensity of KWEW8-DACM at λ<sub>280</sub> and
slightly lower than KWEW8-DACM at $\lambda_{373}$. The fluorescence emission of the DACM dye labeled KWEW8 upon irradiation at 280 nm is around $3.00 \times 10^4$ CPS and $\lambda_{280}$ for the DACM dye labeled KWEW16 is around $5.00 \times 10^4$ CPS. The fluorescence emission of the DACM dye labeled KWEW8 upon irradiation at 373 nm is around $9.00 \times 10^4$ CPS and $\lambda_{373}$ for the DACM dye labeled KWEW16 is around $8.00 \times 10^4$ CPS.

Figures 17 through 19 display the average fluorescence of three different samples of KWEW4 at different concentrations in the micromolar range. The fluorescence measurements were made by adding the designated peptide to Thioflavin T (ThT), and each measurement was made over a specific time interval. Thioflavin dyes are used for biophysical studies of protein aggregation. A strong fluorescence emission is detected when ThT binds to fibrils. Figures 17 through 19 have poor correlation, but each plot has a positive slope providing evidence that fibrillation is occurring with an increase in fluorescence at all three concentrations of the KWEW4 peptide. Figure 20 through 22 display the average fluorescence of three different samples of KWEW8 at different concentrations in the micromolar range. The correlation of the plots in figures 20 through 22 is stronger than the correlation displayed in figures 17 through 19. Figures 20 to 22 also exhibit a positive slope, confirming that fibrillation is occurring with an increase of fluorescence at all three concentrations of the KWEW8 peptide. The average fluorescence emission for KWEW4 and KWEW8 at each designated concentration was obtained at 483 nm.
Future Directions:

**Methods of Energy Transfer**

To expand the scope of this research and to demonstrate the versatility of this approach, a new genetic construct is being prepared. This construct incorporates an Amber Nonsense Codon for site selective replacement of tryptophan with L-(7-hydroxycoumarin-4-yl) ethylglycine. Energy transfer between two spectrally and spatially distinct dyes would provide a platform model for measuring distances which electronic energy may propagate through $\pi-\pi$ interactions. A positive result suggests the peptide-based optical antenna could direct light between and/or around nanoscale building blocks. The resonance energy transfer dynamics will be investigated by the time delay of the fluorescence emission between the two acceptors dyes. This will be done using transient absorption spectroscopy which uses an ultrafast laser and pump probe technology on a femtosecond timescale.
Asymmetrical Dual Dye Polypeptide

After this dual dye polypeptide is made, a genetically engineered polypeptide terminating in Lucifer yellow as a final energy acceptor would be of interest. Two important parameters make this system interesting for academic, technological, and economic reasons. Tryptophan emission does not overlap with Lucifer yellow absorbance. Control of tryptophan- Lucifer yellow separation distances greater than 300 Å is possible. Validation of energy transfer resulting from the superposition of nuclear wave functions could be used in the development of unique optoelectronics and quantum computers. There are two possible modes of energy transfer that can be examined in the asymmetrical dual dye polypeptide system. If energy transfer is quantum coherent the two spectrally and spatially different dyes will fluoresce simultaneously. If energy transfer is quantum incoherent then the L-(7-hydroxycoumarin-4-yl) ethylglycine dye will fluoresce before the Lucifer yellow acceptor dye. Quantum coherence occurs when the dipole propagation during irradiation occurs as a single array. Quantum incoherence occurs where the dipole propagation pattern is not in a single array, instead irradiation occurs in a stepwise manner.
References


