

**Studies of the microstructure of self-assembling peptides:
Effects of chemical cross-linking and growth characterization**

by

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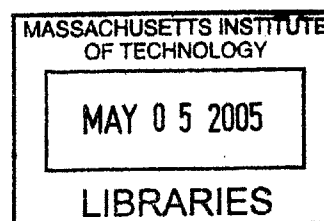
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Abstract

Peptide is a biomaterial with great promise in tissue engineering because it has known to be capable of self-assembly. Also, the peptide scaffolds can support neuronal cell attachment, differentiation, and neuron outgrowth. They might therefore play a role as a substrate for functional synapse formation between neurons.

One important feature of peptide gel is that its modulus can modulate cell motility in combination with the observation that streptavidin and biotin can be used to increase the peptide gel's modulus as measured by rheometry that raises the prospect of controlling cell function by control of matrix physical properties. Atomic force microscope (AFM) was used to reveal the mechanism of modulus change at the nano and microscales. AFM images show that as the amount of biotinylated peptide increases, self-assembly effect is impeded and there are more but smaller aggregates. Streptavidin tethers to regular peptide as well as biotinylated peptide. Also, streptavidin and biotin can cross-link peptide fibrils. These AFM results can explain the increased modulus found by rheometry.

To better describe the growth from individual monomers to filaments, and to exert a measure of control over the nature of network or characteristics of individual filaments requires a better fundamental understanding of the process of filament formation. Therefore, we observed the growth of RAD16- Π by self-assembly in order to gain better understanding of process by which filaments grow. Through a combination of AFM and dynamic light scattering we are able to characterize peptide growth quickly and easily. Dynamic light scattering (DLS) provides a single and convenient means to follow peptide filament growth with time.

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1.Introduction

Nowadays biomaterials like peptide have altered our concept of medical treatment. Because many diseases cannot be treated solely by small molecule drug, cell-based therapy is emerging as an alternative approach. Peptide is one of the biomaterials that show great potential in cell-based therapy because peptide has the following advantages.

First, peptide is known to have self-assembly. Also, it can support neuronal cell attachment, differentiation and synapse formation. In addition, we can control physical properties of peptide gel very easily. And the important point is that physical properties of gel can influence many factors of cell function. One of them is peptide gel's modulus that is known to be related to cell's motility. That is to say, at low modulus substrate cells move around, while at high modulus substrate cells cannot move. Therefore, by control of stiffness of peptide gel we can modulate cell motility. This is one of my general motivations. In order to increase the modulus of peptide gel streptavidin and biotin were added into peptide solution because they are known to have a strong binding. To explain the increased modulus, the cross-linked peptide gel was imaged by use of AFM. We found out the possibilities of cross-linking based on AFM images.

An alternative was devised to characterize peptide growth. To understand the fundamental process of peptide filament growth demands observation of peptide growth. In combination with AFM, dynamic light scattering (DLS) can be useful to see peptide growth because its operation is very simple compared to AFM. Based on diffusion coefficients measured by DLS, the average lengths were calculated with time to characterize peptide filament growth.

2.Experimental methods

Rheometric measurements were made using cross-linker to increase modulus and then she measured modulus by use of rheometer that is typically used by many people (Kim, 2004). The result was the increased modulus. However, rheometry shows only bulk characteristics and the increased modulus might be due to peptide growth. Therefore, we want to see what really changed the modulus at the micro and nano scale. That is why the experiment was started with Atomic Force Microscopy (AFM). In order to see at the nano scale, two samples were imaged with cross-linker and without cross-linker and then they were compared.

An alternative measurement method was developed to measure peptide growth by use of dynamic light scattering (DLS). People usually use AFM to see peptide growth. But, AFM demands very careful effort to get good image. On the contrary, DLS is very easy to operate and we can figure out peptide growth more quickly and easily compared with AFM. With this alternative the fundamental process of peptide filament growth was understood.

Streptavidin preparation

S treptavidin was ordered from Molecular Probes (Eugene, OR, USA) and the powder was stored at -20 °C. Streptavidin solution was prepared by adding ultra-filtered water to 0.4mg of streptavidin to bring the concentration to 0.4mg/mL and the solution was vortexed at 200 rpm for 1 min. After 10 hours the sample was diluted by 300 folds and then imaged by use of AFM.

Peptide synthesis and sample preparation

The peptides RAD16- Π and b-RAD16- Π , of sequence [COCH₃]-RARADADARARADADA-[CONH₂] and [COCH₃]-K(biotin)-GGGGG-RARADADARARADADA-[CONH₂] respectively (R is arginine, A is alanine, D is aspartic acid, G is glycine), was custom-synthesized from Synpep(Dublin, CA, USA) and the lyophilized powder was stored at 4 °C. Peptide solutions were prepared by adding HCl solution (pH ~ 3.45) to roughly 1mg of peptide powder to bring the concentration to 1 mg/mL (0.584mM). The mixture was then vortexed at 2000 rpm for 1 min, after which the peptide was in solution. Then, the solution was divided into two equal parts (each 500 μ L) and filtered by use of a centrifuge (3000 rpm, 2 min) and centrifuge tube filters that have pores of which size is about 200 nm. The pH of filtered solution was about 3.00.

To investigate cross-linking, two samples were prepared, regular RAD16- Π and biotinylated-RAD16- Π at a concentration of 1 mg/mL. After vortexing at 2000 rpm for 1 min, they were mixed with the amount ratio changed from 100%-biotinylated RAD16- Π to 1%-biotinylated RAD16- Π . Then, the mixed samples were vortexed again at 2000 rpm for 1 min and sonicated for 10 min. To remove aggregates, the samples were filtered through the same centrifuge tube filters. Then, streptavidin solution was added into each biotinylated peptide solution. The ratio of streptavidin and biotin was 1:150.

By use of adhesive tape mica surfaces have been freshly cleaved, then immediately aliquots of 10 μ L were removed from each peptide solutions and put on mica surfaces after each sample's preparation. The mica surface was then rinsed with

100 μ L of HCl solution of pH \sim 3.45, and it took about 5 min to dry the rinsed surface in air.

There were a lot of peptide aggregates in peptide samples; therefore, the atomic force microscopic image quality was initially not good to analyze. To remove the aggregates, ultrasonic cleaners were tried. When ultrasonic energy is introduced into a cleaning solution, cavitation, the foundation of ultrasonic cleaning occurs. Ultrasonic energy causes alternating patterns of low- and high-pressure phases. During the low-pressure phases, minute bubbles, or vacuum cavities form. During the subsequent high-pressure phases, the bubbles implode violently. This is called cavitation. The bubbles are small enough to penetrate even microscopic crevices, cleaning them thoroughly and consistently.

Sample imaging by Atomic force microscopy (AFM)

The surface images were obtained by use of an atomic force microscope (Multimode AFM, Digital Instruments, Santa Barbara, CA, USA) that scans the mica surface in air operating in Tapping Mode. Another mode, contact, was not a good way to image soft materials like peptide because it can distort and cause damages on the peptide samples.

Also, in order to minimize the tip tapping force, FESP tips were used with spring constant of 1-5 N/m and tip radius curvature of 5-10nm. 512 x 512 pixels were taken for high resolution and AFM produces topographic images in which brightness is a function of height. The parameters were as follows: the tapping frequency is about 70kHz, integral and proportional gains are 0.2-0.4 and 0.3-1 respectively, setpoint 0.5-0.8, scanning speed 1 Hz. The images were saved after completing the scanning.

Image analysis

In order to measure sizes of fibers, Nanoscope III software(version 4.42) was used to analyze AFM images. The diameter and height were directly measured from images. Comparing a molecular model of RAD16- II , overestimation effect due to the finite tip size was considered when the diameter was measured.

Characterization of filament length by Dynamic light scattering

The experiment was carried out by use of PD2000DLS made by Precision detectors, Inc. (Franklin, MA, USA).

This DLS was used in two ways. One was to measure the changed concentration of filtered samples, and the other was to measure average length of peptide filament length.

Filtering is necessary for AFM images of good quality; however, this causes the concentration change of peptide sample. DLS can one of methods to measure this changed concentration due to filtering.

There are various ways to characterize peptide growth including AFM that is difficult to operate. Instead of using the difficult method, it is better to use an easier way to see peptide growth. 200 μ L solution was placed into a transparent specimen bottle and the decreasing diffusion coefficient had been measured for 50 hours(See 4.5 for a description of the method of DLS analysis).

3.Results

3.1 Structure of RAD16- Π fibers

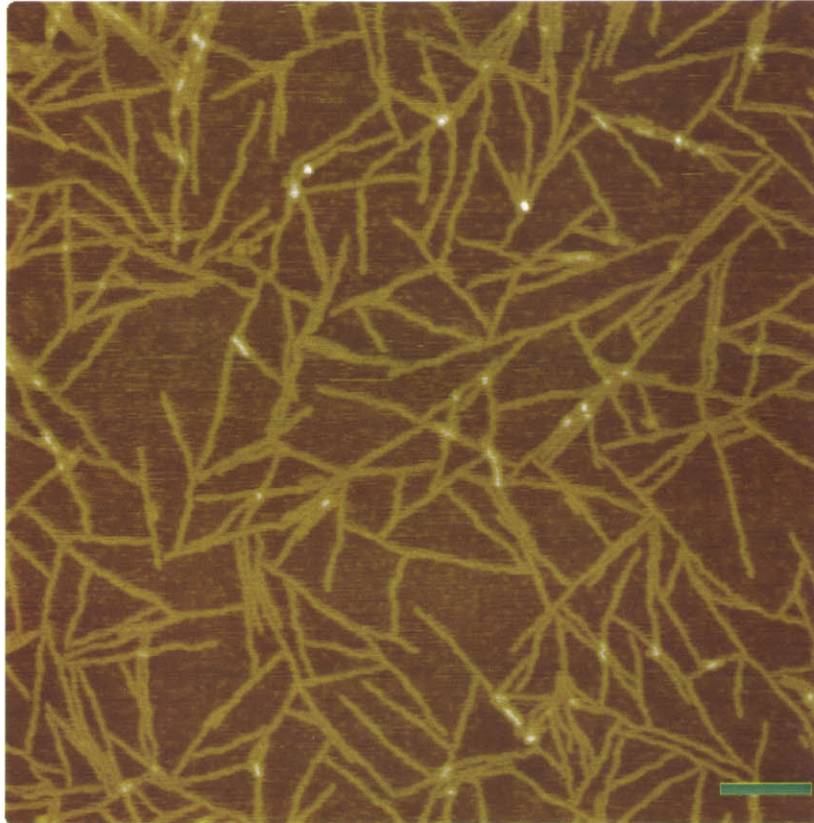
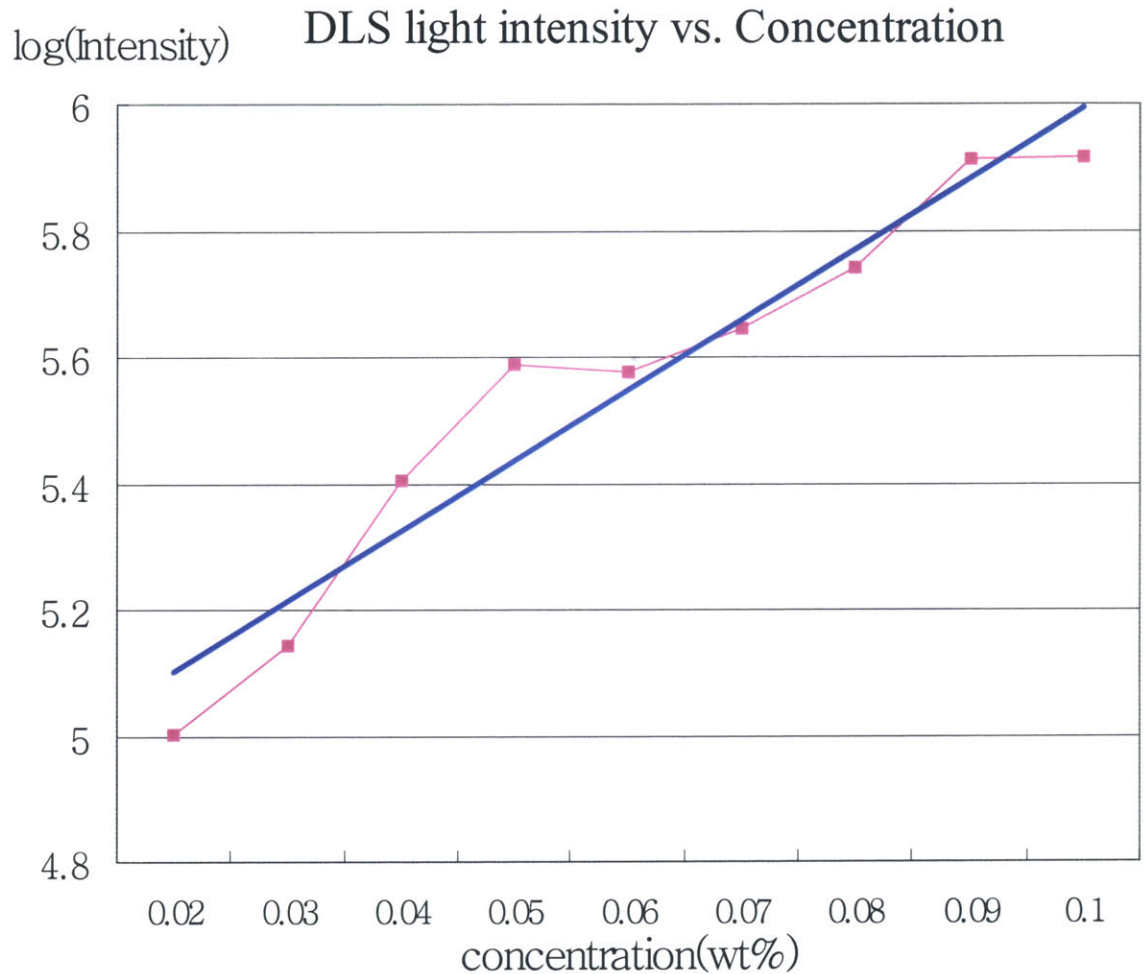


Figure 1. AFM scan of fibers formed by the peptide RAD16- Π in 1mg/mL aqueous solution at room temperature. The sample was allowed 30 min of self-assembling time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

AFM produces peptide structure on mica surface in air. Even though this is not a physiological condition, we can successfully analogize peptide fiber structure and formation based on AFM images. There are a lot of fibers with branches. To explain self-assembly way of RAD16- Π , the width and height were measured. Also, we can see many peptide branches.

3.2 Concentration determination



Graph 1. The graph of changed concentrations vs. log (DLS intensity)

Due to filtering to remove peptide aggregates, the concentration was changed; therefore, it is necessary to measure the exact concentration after filtering. One of the methods to measure concentration can be dynamic light scattering. It is hypothesized that the concentration is just proportional to log (scattered intensity). First, the samples with various concentrations were prepared, and then the scattered intensities were

measured by use of dynamic light scattering. The results are the pink line of graph 1.

This line was calibrated by blue line that is

$$y = 11.1582 x + 4.8783 \quad (1)$$

where y is $\log(\text{scattered intensity})$, x is concentration

Since post-filtered sample's concentration is 200000 counts/sec, the concentration is about 0.038wt% based on equation (1).

3.3 Streptavidin image

Before adding streptavidin into peptide solution, only streptavidin was imaged because it was necessary to distinguish streptavidin from peptide filaments. The size information of streptavidin enables us to determine whether peptide fibers are cross-linked or not.

0.4mg streptavidin was mixed with water 100 μ L; therefore, 0.4wt% streptavidin solution was prepared and then the solution was vortexed for 1 minute. After 6 hours the solution was diluted by mixing the solution 5 μ L with water 1495 μ L(300 times dilution) and then imaged following the same procedure as RAD16- II (See Figure 2). Then, the width was measured as 15nm~5nm and height about 4nm.

3.4 Cross-linked structure of RAD16- II by biotin and streptavidin

To explain the increased modulus in rheometry experiment, it is necessary to see what changes the modulus at the micro and nano scales. There are various ways to see peptide nano structure including electron microscopy. But, the resolution of scanning electron microscopy is approximately 10nm that is beyond the width of peptide fiber

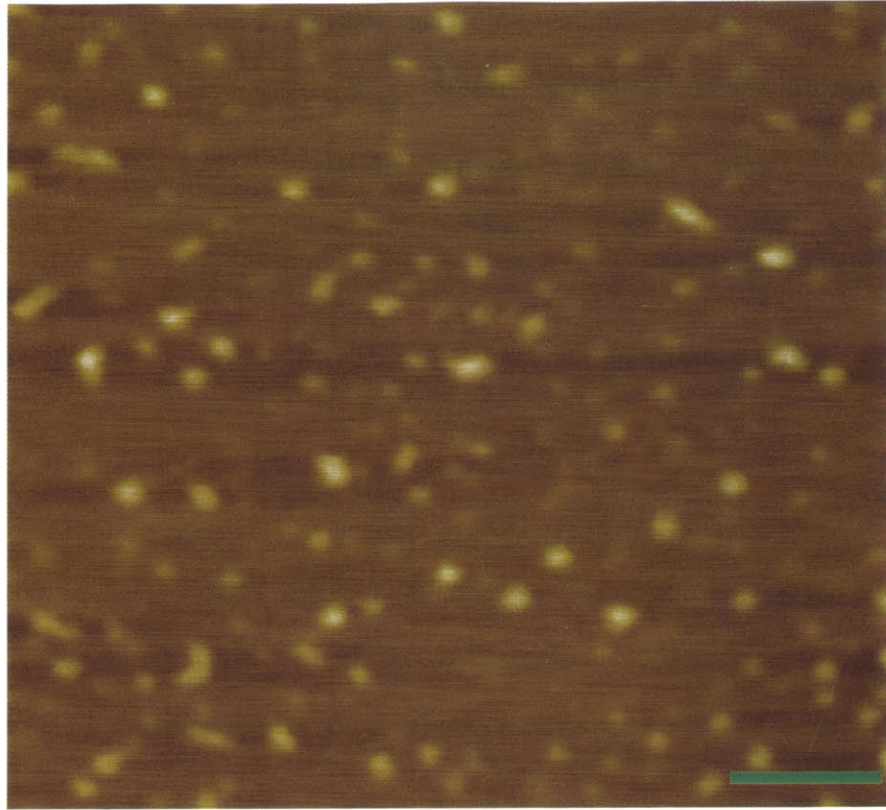


Figure 1. AFM scan of particles formed by streptavidins in 0.4mg/mL aqueous solution at room temperature. The sample was allowed 10 hours of self-assembling time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

and streptavidin. AFM has a resolution of 1nm in tapping mode that enables us to see the nanostructure of peptide fibers.

We used streptavidin and biotine that are well known cross-linkers in biology. Gina Kim measured modulus when she added streptavidin and biotin into RAD16- Π solution. The result was the increased modulus on the order of magnitude. To explain this increased modulus, we imaged streptavidin and various biotinylated RAD16- Π samples on the mica surface to get the definite evidence of cross-linking. First, 100%-biotinylated RAD16- Π was tried (see Figure 3). The sample had been prepared in the

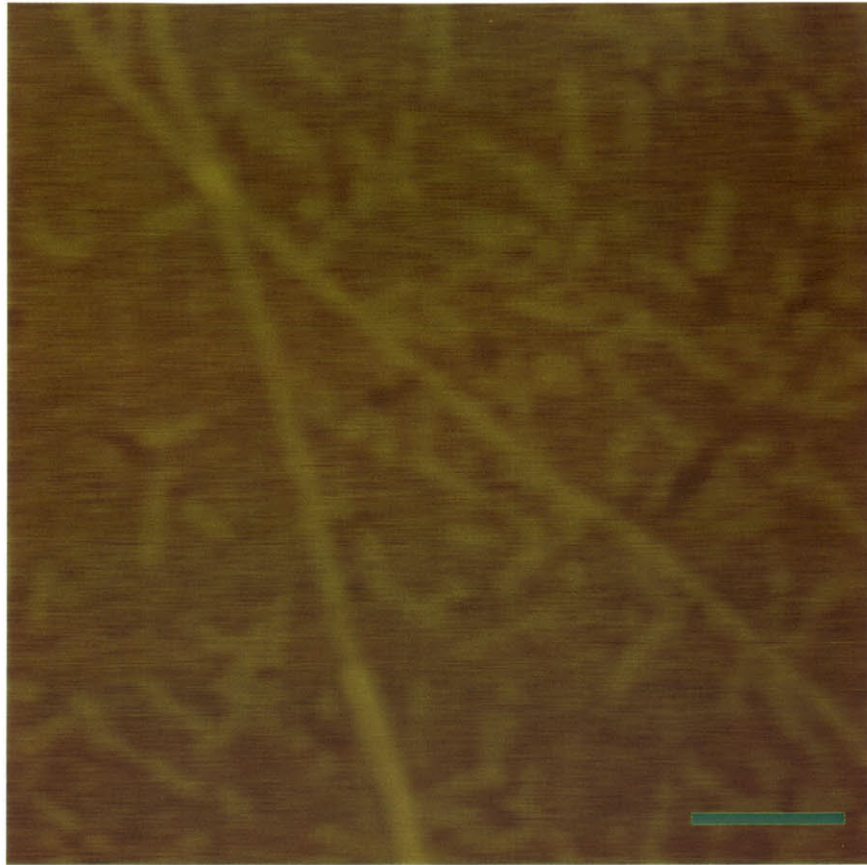


Figure 3. AFM scan of fibers formed by the peptide 100%-b RAD16- II in 1mg/mL aqueous solution at room temperature. The sample was allowed 80 hours of self-assembling time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

same way as regular RAD16- II and incubated for very long time like 80 hours, and then it was imaged by use of AFM. The result was Figure 3. Unlike regular peptide, there are few peptide filaments.

Then for cross-linking, streptavidin was added into the same 100%-biotinylated solution at 78 hours after filtering, and at 80 hours it was deposited on the mica surface and imaged by AFM (See Figure 4). There were a lot of aggregates in the solution

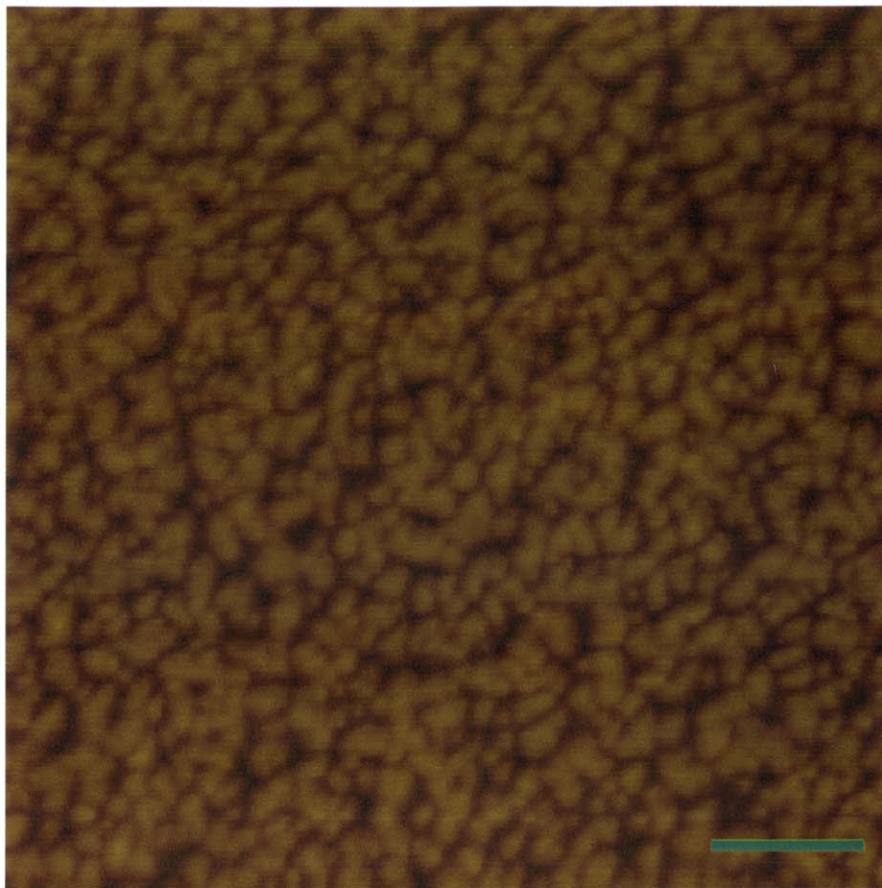


Figure 4. AFM scan of fibers formed by 100%-b RAD16- Π and streptavidin by ratio of S:B~1:150 in 1mg/mL aqueous solution at room temperature. Streptavidin was added into 100%-b peptide at 78 hours after the peptide solution was prepared. Then, the sample was allowed 2 hours of cross-linking time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

instead of cross-linked fibers.

Next, the amount of biotinylated peptide was decreased to 50% for a definite cross-linking image. The sample was deposited on mica surface at 6.5 hours after filtering and immediately imaged by use of AFM (Figure 5). We could see some fibers forming

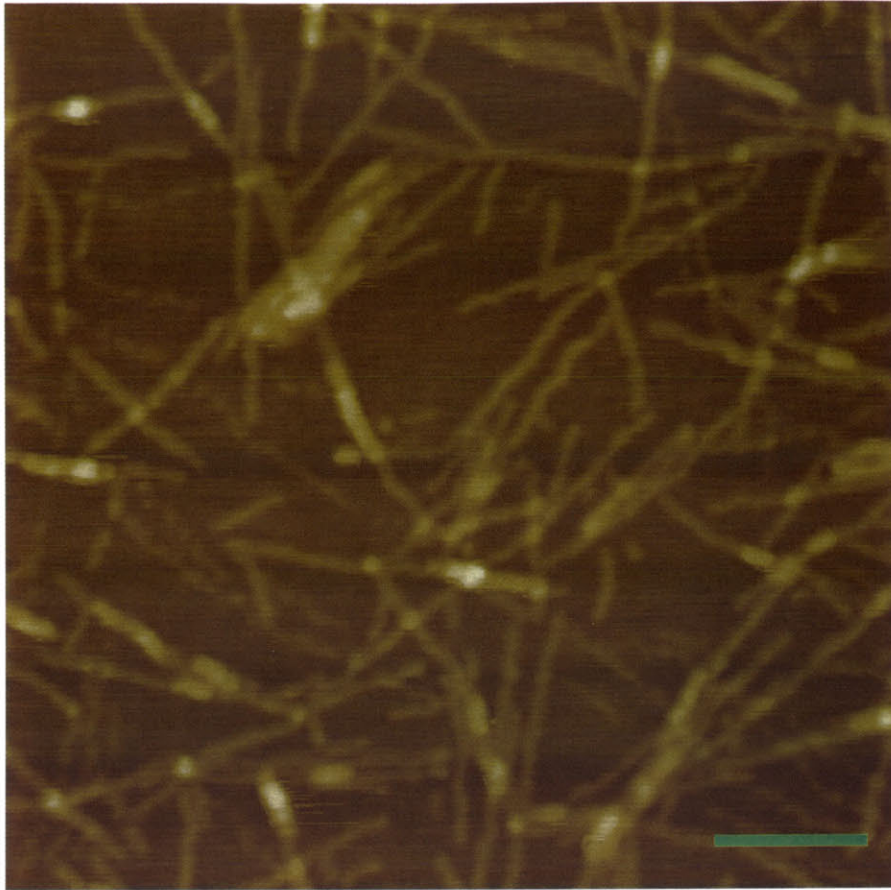


Figure 5. AFM scan of fibers formed by the peptide 50%-b RAD16- II in 1mg/mL aqueous solution at room temperature. The sample was allowed 6.5 hours of self-assembling time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

from self-assembly with short length. Also there were some white positions that could be considered as the overlapping of fibers or peptide aggregates in the same way as regular RAD16- II .

Also, streptavidin was added into 50%-biotinylated RAD16- II solution at 4.5 hours after filtering. The ratio of streptavidin and biotin was 1:150. The sample was imaged at 6.5 hours (Figure 6). There were still many aggregates that prevent analyzing AFM images. In 50%-biotinylated peptide, we can see some fibers unlike

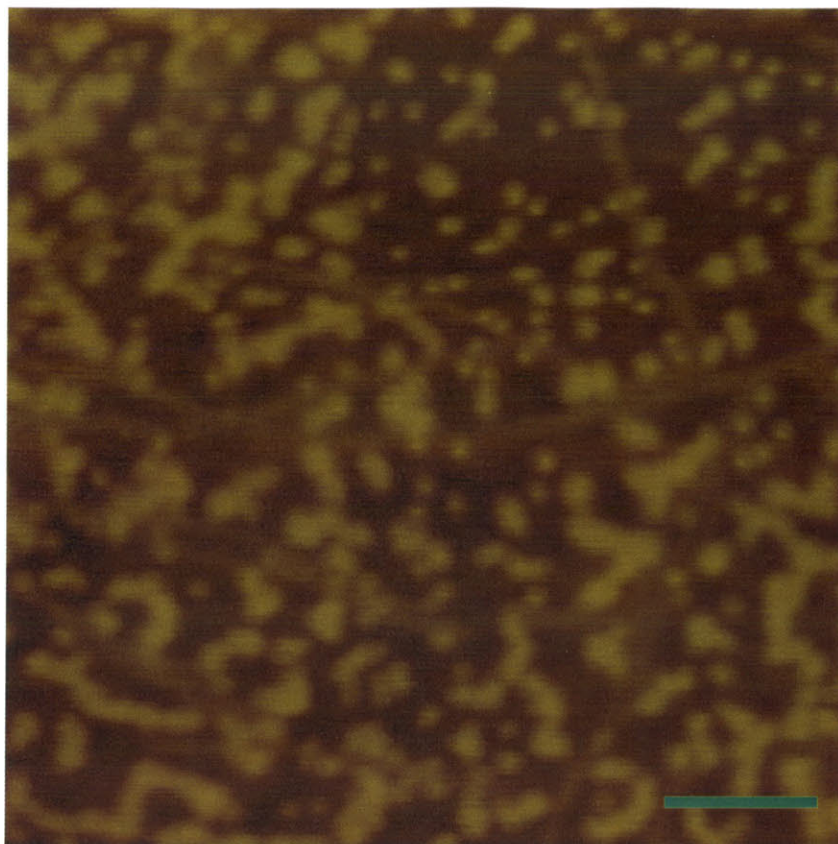


Figure 6. AFM scan of fibers formed by 50%-b RAD16- Π and streptavidin by ratio of S:B~1:150 in 1mg/mL aqueous solution at room temperature. Streptavidin was added into 50%-b peptide at 4.5 hours after the peptide solution was prepared. Then, the sample was allowed 2 hours of cross-linking time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

100% biotinylated peptide. But, it seems that self-assembly effect is small compared with cross-linking effect because there are a lot of biotins on peptide fibers.

Next 1%-biotinylated RAD16- Π was imaged. The result was Figure 7. The image was taken at 6.5 hours after the sample was filtered. This image is almost the same as regular RAD16- Π . The images have long fibers with many branches and white positions. To cross-link peptide fibers, streptavidin was added into 1%-b

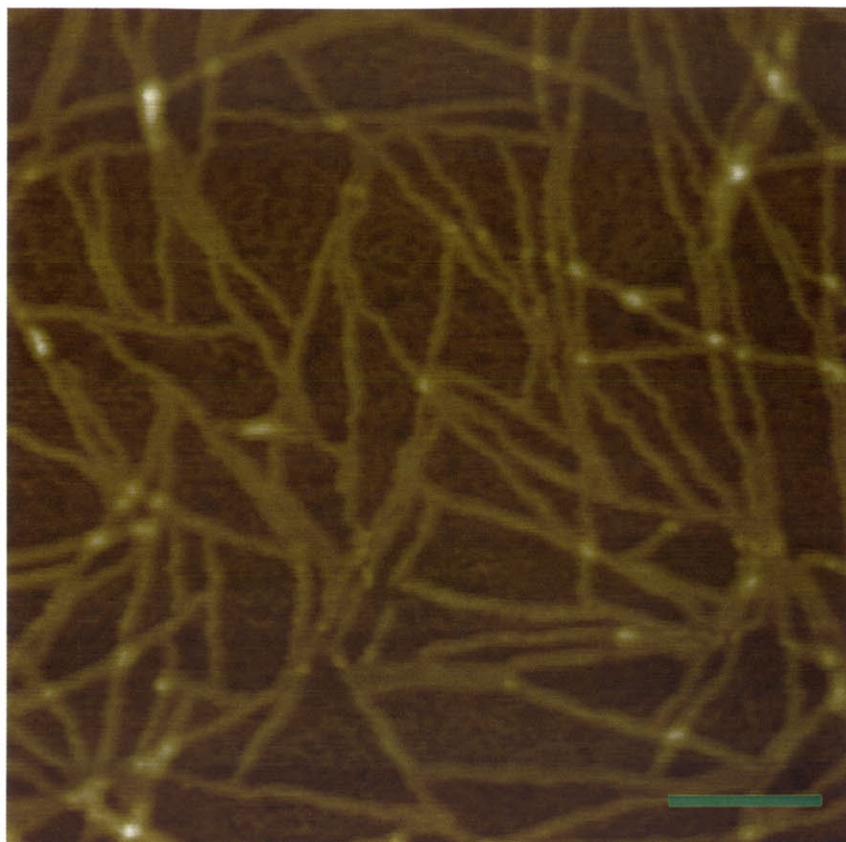


Figure 7. AFM scan of fibers formed by the peptide 1%-b RAD16- Π in 1mg/mL aqueous solution at room temperature. The sample was allowed 6.5 hours of self-assembling time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm

RAD16- Π (Figure 8). At 4.5 hours after filtering, streptavidin solution was mixed with 1%-biotinylated RAD16- Π . The ratio of streptavidin to biotin is 1:150. At 2 hours after that, the solution was deposited on mica surface and the sample was imaged by use of AFM. Based on the differences between Figure 7 and Figure 8, we can observe the tendency for cross-linking. Gina Kim added streptavidin into regular RAD16- Π (Regular means no biotin in peptide solution). Then, she measured modulus by use of rheometer that is typically

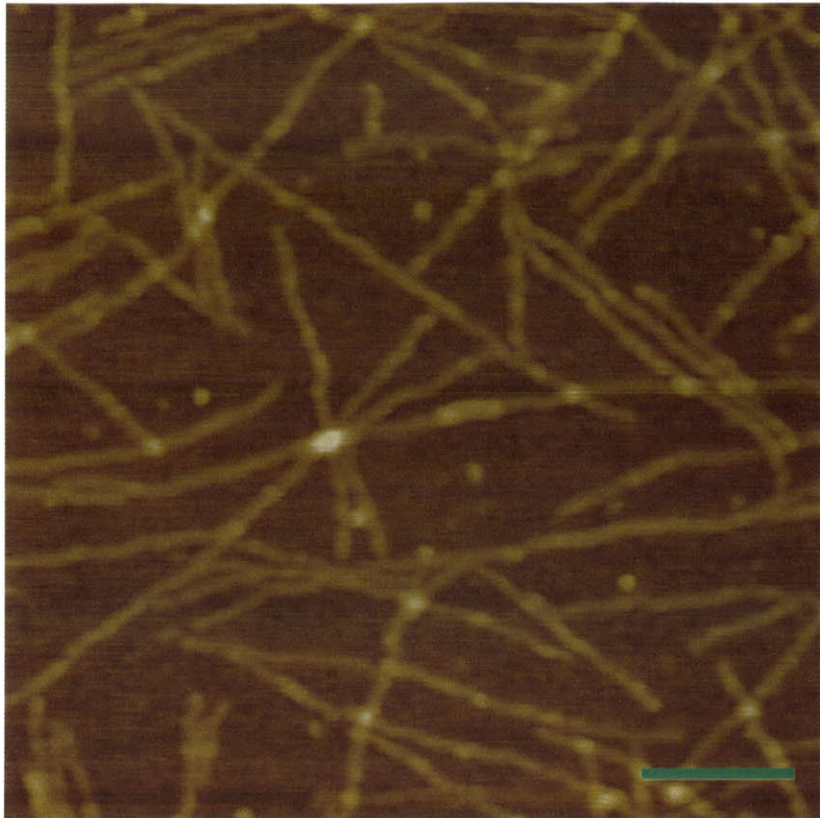


Figure 8. AFM scan of fibers formed by 1% RAD16-II and streptavidin by ratio of S:B~1:150 in 1mg/mL aqueous solution at room temperature. Streptavidin was added into 1% peptide at 4.5 hours after the peptide solution was prepared. Then, the sample was allowed 2 hours of cross-linking time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

used by many people. The result was the increased modulus on the order of magnitude. In an attempt to explain this increased modulus, the sample of regular peptide with streptavidin was imaged by use of AFM. The results are shown in Figures 9 and 10. At 4.5 hours after filtering, streptavidin was added into regular peptide and the sample was imaged at 6.5 hours after filtering. There are a lot of particles on the surface. These particles do not seem to be attached on peptide fibers.

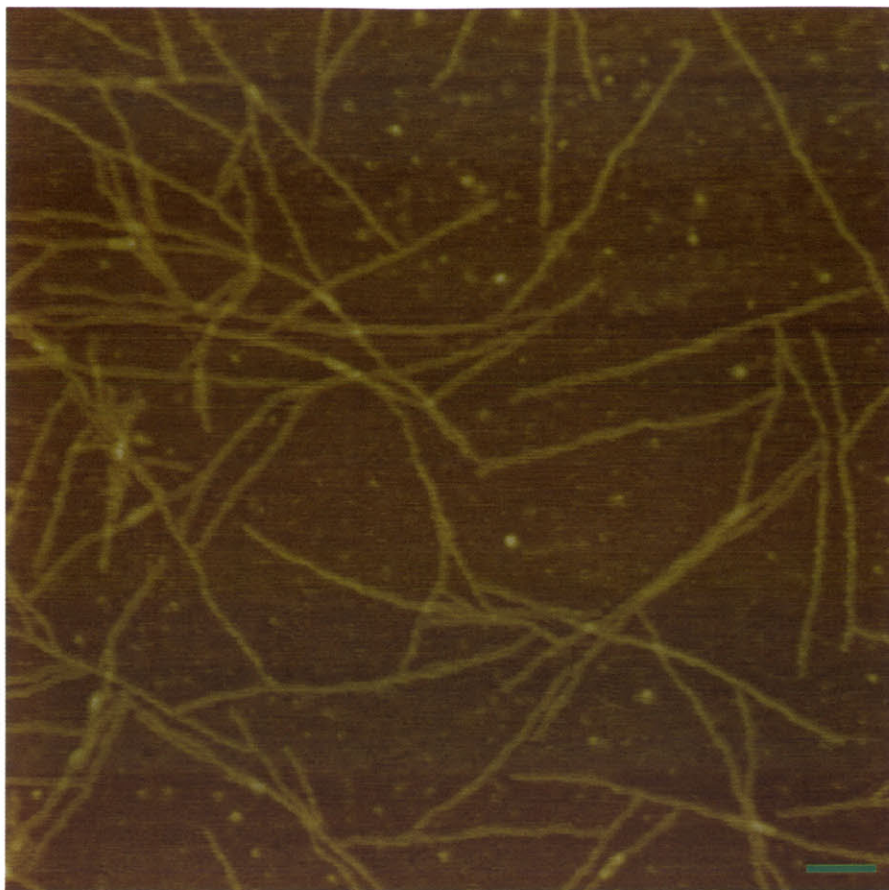


Figure 9. AFM scan of fibers formed by 0%-b RAD16- Π and streptavidin in 1mg/mL aqueous solution at room temperature. The same amount of streptavidin as that of 1%-b peptide was added into 0%-b peptide at 4.5 hours after the peptide solution was prepared. Then, the sample was allowed 2 hours of cross-linking time, after which it was deposited on a mica substrate. The surface was then rinsed with HCl solution and dried in air. The brightness of feature increases as a function of height and the scale bar is 100nm.

For more exact information about attaching of streptavidin on peptide fibers, another image was taken at a different position in the same sample (Figure 10). Most particles do either not attach on peptide fibers; but some particles seem to attach on peptide fiber in Figure 10.

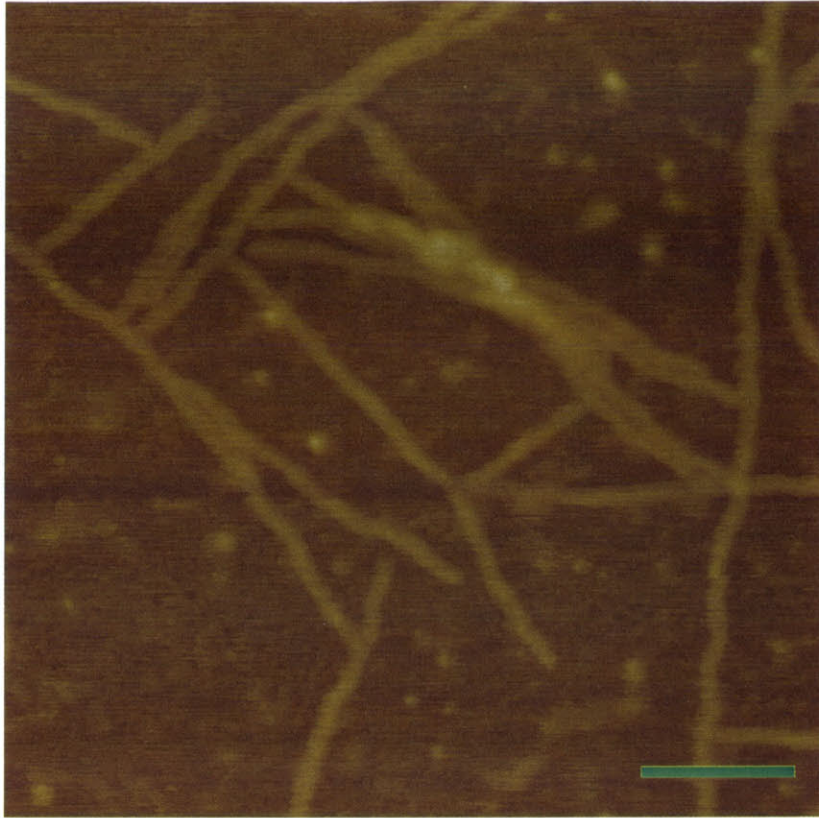


Figure 10. The same sample as Figure 9, but different position image

3.5 Dynamic light scattering

3.5.1 Basic principles of DLS

To characterize peptide growth, an alternative was devised. As peptide fibers grow, the scattered beam is changed. The scattered intensity is transformed into the

$$G(\tau) = \lim_{T \rightarrow \infty} \left[\frac{1}{T} \int_0^T i_{\theta}(t) i_{\theta}(t + \tau) dt \right] \quad (2)$$

correlation function by DLS, and then the function is normalized by the initial value.

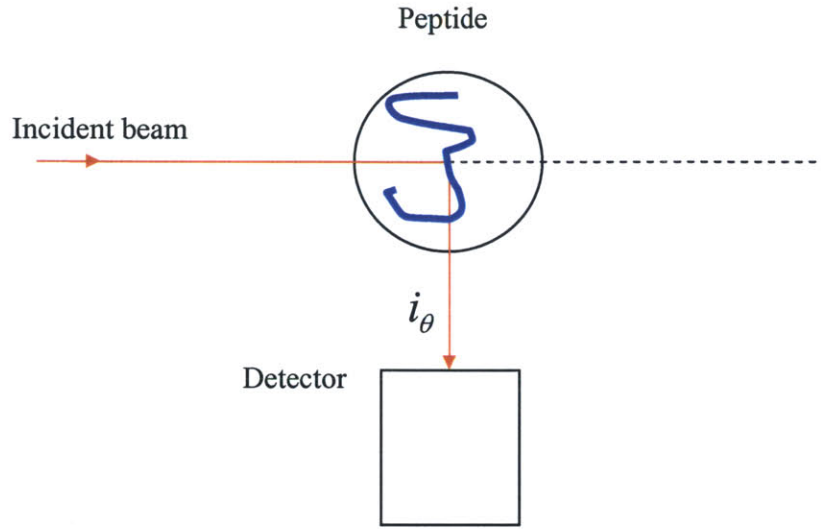


Figure 11. DLS basic principle

$$g(\tau) = G(\tau) / G(0) \quad (3)$$

We can get the mean decay rate fitting the normalized correlation function with

$$g(\tau) = \sum C_i \exp(-\Gamma_i \tau) \quad (4)$$

exponential functions.

where C_i : the fractional weighting factor for species i

Γ_i : the characteristic decay rate for species i

The mean decay rate is calculated as

$$\bar{\Gamma} = \sum C_i \Gamma_i \quad (5)$$

, and it determines diffusion coefficient as follows.

$$D = \frac{\bar{\Gamma}}{q^2} \quad (6)$$

where q : scattering vector's magnitude

In order to measure this diffusion coefficient of RAD16-II filaments, the same sample as AFM experiment was prepared. 0.1wt% sample was prepared, and then it was vortexed for 1 min at 2000 rpm. After sonicating for 20 minutes, the sample was

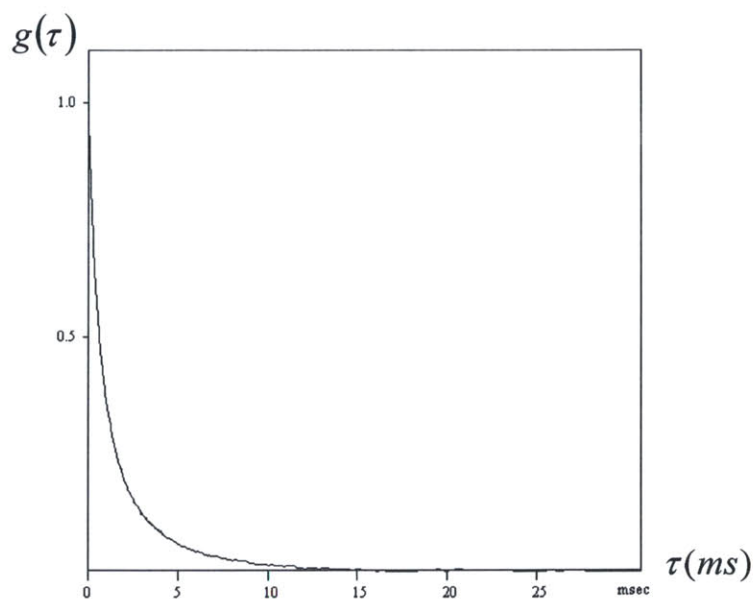


Figure 12. Normalized correlation function

filtered through pores. Then, the diffusion coefficient was measured from 0 hour to 50 hours in order to characterize peptide filament growth (See Figure 13).

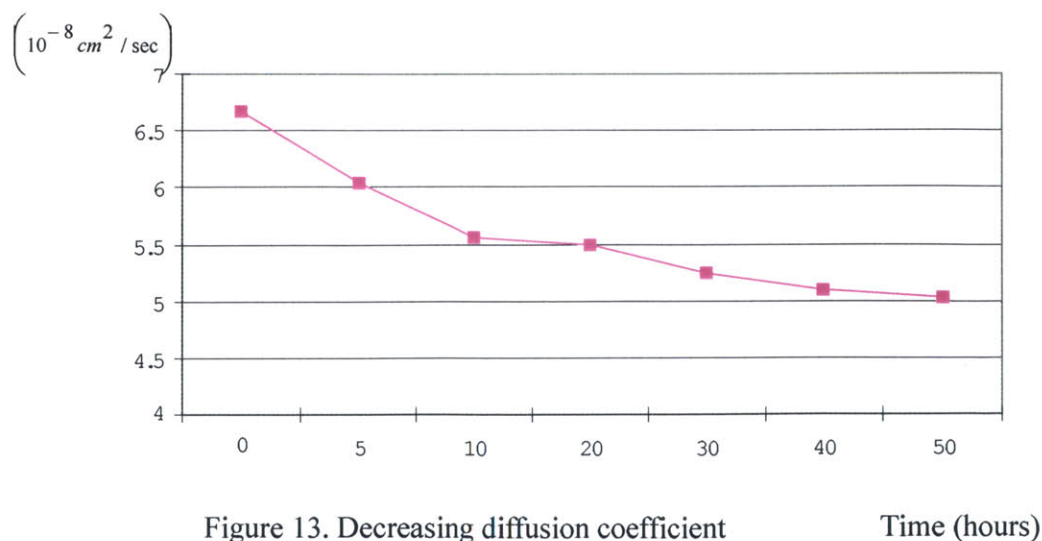


Figure 13. Decreasing diffusion coefficient

Time (hours)

4. Discussion

4.1 Structure of RAD16- II

The motif, RAD, was incorporated to mimic the known cell-adhesion motif RGD that is found in many extracellular matrix. We changed the modulus like I and II. My part is modulus II. The basic units of RAD16- II are arginine(R), aspartic acid(D), and alanine(A). Arginine and aspartic acid are hydrophilic, while alanine is hydrophobic.

It has an alternation like hydrophilic, hydrophobic, hydrophilic, and hydrophobic. Alsoit has a polarity from the terminal amide group to the terminal carbonyl group. This hydrophobic effect and polarity are known to be important in self-assembly of peptide. Based on a molecular model, I calculated a chain's width and height. The height is about 1.2 nm and the width is about 6.6nm. These would be important in analyzing AFM images.

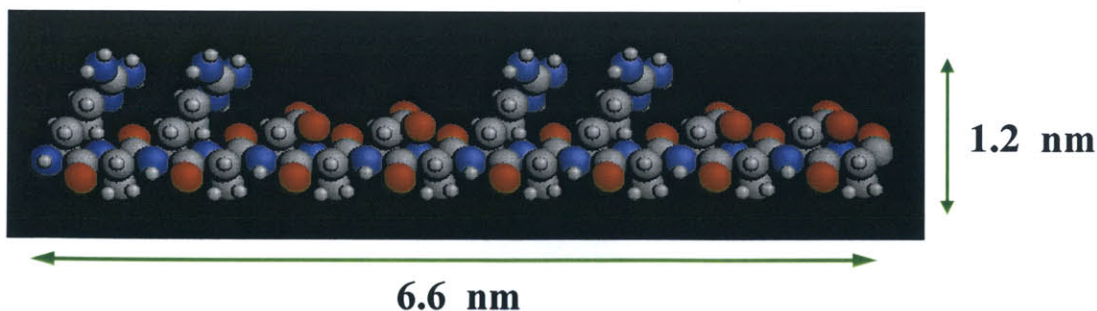


Figure 14. Molecular model of RAD16- II

Self-assembly of another peptide, KFE8, is known well. Here I hypothesize that RAD16- II self-assembles in the same way as KFE8. This is the starting point for my research. Based on the AFM images, I will verify self-assembly of RAD16- II .

In an aqueous solution, RAD16- Π has hydrophilic side groups and hydrophobic side groups. The hydrophobic side groups shield themselves from water; therefore, double layers form. Also, to stabilize the structure, anti-parallel β -sheet structure form because there are a lot of carbonyl group and amide group to form H-bonding in the peptide chain. Hence, double layers with anti-parallel β -sheet structure form peptide fibers. This is baseline for my research.

To see peptide structure, AFM was used. The basic principles are like this. While a tip scans a surface, a cantilever beam deflects. Therefore, a laser beam also deflects. From the laser beam deflection, AFM produces topographic images that tell us about sample's height and width. This would be important in proving self-assembly way and cross-linking of peptide. In addition, peptide gel is very soft material (Young's modulus of peptide is on the order of kPa.), thereby, tapping mode was used instead of contact mode to reduce lateral forces and prevent damages on samples.

There are a lot of peptide fibers with branches on the surface (See Figure 1). These fibers have branches unlike KFE8. Also, the white positions can be considered as the overlapping or nucleates of fibers. To prove the double layer theory of RAD16- Π , the width and height were measured. The width was about 8nm and height 2nm (See Figure 15)



Figure 15. Average width and height of RAD16- Π fiber

Considering the monolayer of which height is about 1.2nm(See Figure 14), it is reasonable that peptide fibers compose of double layers because the measured height is about 2nm. But, the widths are different. Based on the molecular model, the actual width is about 6.6nm;however, the measured width was about 8nm. To explain the difference, we can consider AFM tip's radius curvature that is about 5nm(See Figure 16)

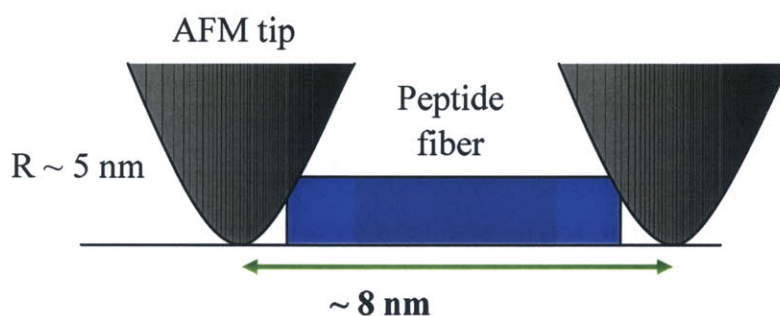


Figure 16. The measured width considering AFM tip's radius curvature

4.2 Concentration determination

The peptide sample has a lot of peptide aggregates; thus, the image quality was not good to analyze. To remove the aggregates, the samples were filtered through pores of which size was about 200nm. Then, in order to measure the changed concentration, dynamic light scattering was used for the sample with various concentrations. The result is graph 1. As you see, the changed concentration was about 0.038wt% from the calibration line.

4.3 Streptavidin image

Before imaging cross-linked peptide fibers, only streptavidins were imaged because it was necessary to see streptavidin's shape and size (See Figure 2).

The particle's width is about 15~5nm and height 4~1nm. Considering that the actual molecular width of streptavidin is about 10nm and AFM tip's radius curvature that is about 5nm, the particles has the size comparable to streptavidin. The size variance may be due to imaging streptavidin at the dry state

4.4 Cross-linked structure of RAD16- II by biotin and streptavidin

First, 100%-biotinylated peptide was tried (See Figure 3). Even though the sample was incubated for a very long time like 80 hours, it was very difficult to see peptide fibers. This may be due to too many biotins on peptide fiber. Also, when streptavidin was added into 100%-b-peptide, many aggregates form (See Figure 4).

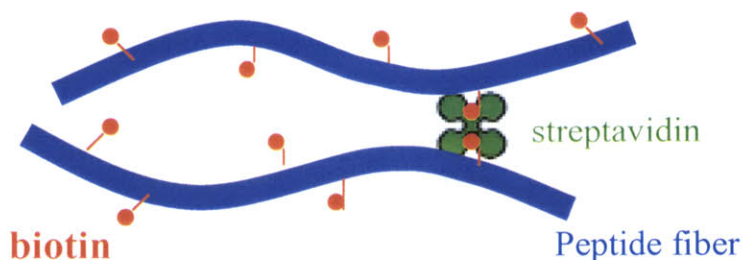


Figure 17. Cross-linked peptide fibers

Considering the shape and size of aggregates these aggregates may be due to strong binding between strepavidin and biotin and four binding sites of streptavidin.

There was no definite cross-linking image in case of 100%-b-peptide; therefore, the amount of b-peptide was decreased to 50%(See Figure 5). Some fibers form; but they are short compared with regular peptide image. Also, when streptavidin was added, there were still many aggregates (See Figure 6). It was difficult to find out definite cross-linking. Hence, 1%-b peptide was tried (See Figure 7). Figure 8 was taken when streptavidin was added into 1%-b peptide. Based on the differences between Fig.7 and Fig.8, there will be explanation for cross-linking.

First, seeing peptide fibers in Figure 8, there are a lot of particles on peptide fibers. These particles have the size of streptavidin(The size of streptavidin is based on the image of only streptavidin(See Figure 2)).

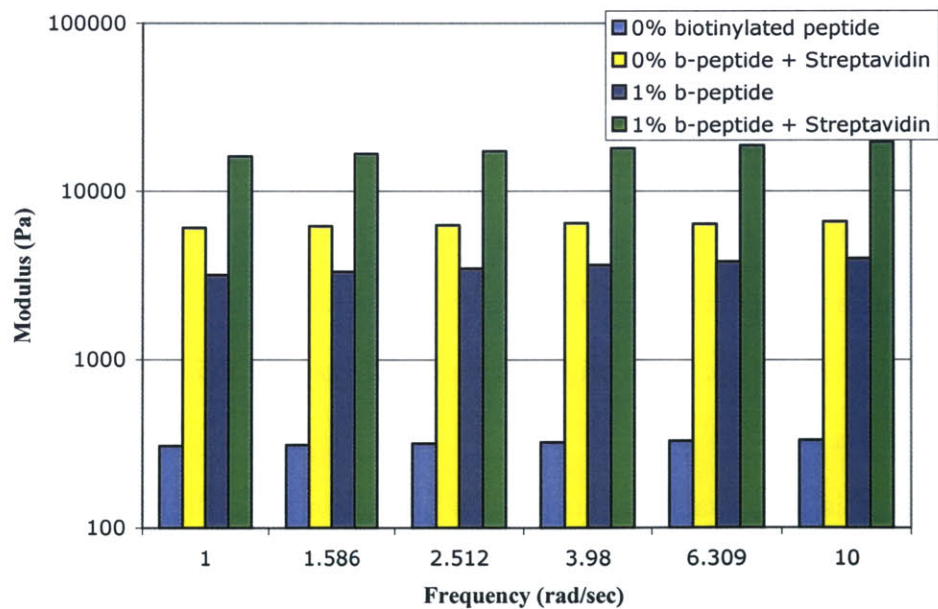
Also, considering the distance between the particles, that was about 30~40nm(See Figure 8). In case of 1%-b peptide, the theoretical average distance between biotins is about 50nm. Therefore, the positions of particles can be the binding sites of streptavidin and biotins.

Seeing the up-zoomed figure, we can figure out peptide cross-linking more clearly. There is a particle between fibers. This particle has the size of streptavidin. Also, due to the binding, the fibers seem to be curved. In addition, this image was taken in diluted state. Therefore, in the original solution, streptavidin and biotin can cross-link peptide fibers.

But, it is not certain that streptavidin and biotin are really cross-link peptide because AFM gives only morphological information. Also, only one possible cross-linking point was found out. It could happen by chance. This is the limit of AFM. However, AFM shows the possibility of cross-linking.

Gina Kim measured the storage modulus of the regular RAD16-II mixed with

streptavidin. The modulus was increased on the order of magnitude. To explain the increased modulus, the regular peptide with streptavidin was imaged (See Figure 9 and 10). Most streptavidins do not attach on regular peptide fibers because there is no biotin on peptide fibers. But, some streptavidins seem to be attached on regular peptide fibers (See Figure 10). This is consistent with rheometer results of Gina Kim (See Graph 2). In both cases of 0% and 1%-b peptide, storage modulus was increased on the order of magnitude from 300 Pa to 6000 Pa and from 3000Pa to 18000Pa, respectively. To explain these increased modulus, it is reasonable that streptavidin and biotin are cross-linking peptide fibers.



Graph 2. Storage modulus of regular and cross-linked RAD16- II

4.5 Dynamic light scattering

The decreasing diffusion coefficient was measured for 50 hours (See Figure 13). This means more resistance to the diffusion of peptide fibers. Since viscosity of solvent is constant with time, this decreasing diffusion coefficient can be related to increasing size of peptide fibers.

Based on the assumptions that a peptide fiber is a cylinder of length L and radius R and that there is no electrolyte friction effect, there is a well-known equation to relate diffusion coefficient to L and R .

$$D = \frac{k_B T}{3\pi \eta L} \left(\ln\left(\frac{L}{2R}\right) + 0.312 + 0.565 \times \left(\frac{L}{2R}\right)^{-1} + 0.1 \times \left(\frac{L}{2R}\right)^{-2} \right) \quad (7)$$

Where k_B : Boltzmann constant

T : absolute temperature

η : Viscosity of solvent

Assuming that the diameter is about 5 nm based on AFM image and then the length L was calculated for 50 hours based on the decreasing diffusion coefficient measured from dynamic light scattering (See Figure 18)

We can see that peptide fibers grow from 300nm to 400nm for 50 hours. Time was counted after the sample was filtered. It took 2 and 10 min to filter the sample and to measure the diffusion coefficient by use of DLS, respectively. During this time, peptide fiber grows; therefore, DLS can show the average length of 300nm at 0 hour. This result can be compared with AFM results; however, AFM sample is not an original solution; this means that peptide was rinsed by water and dried for imaging on mica surface. Therefore, the AFM image can show a different state than the original solution. Also, it is very tedious to calculate the average length based on AFM image

because we do not know the exact length of fibers at the boundary of image. Hence,

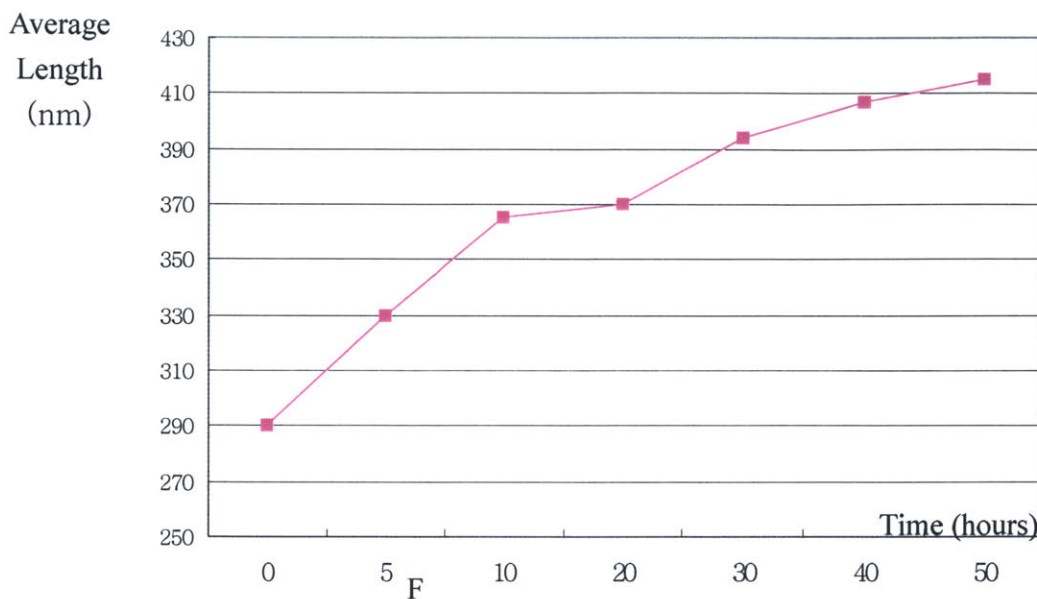


Figure 18. RAD16-II growth

rather than using AFM image to calculate the average length, it can be better to use dynamic light scattering to characterize peptide growth.

4.6 The nature of branching

Unlike KFE8 RAD16-II has a lot of branches. To explain branching, there are two possibilities (See Figure 19). One is that peptide chain is wide enough to be divided maintaining H-bonding between peptide chains (Figure 19a). Another possibility seems irrelevant to H-bonding (Figure 19b). It is guessed that this possibility can be related to hydrophobic effect. More experiments are necessary to check this assumption. One possible experiment can be to change the number of sequence of RAD like RAD8.

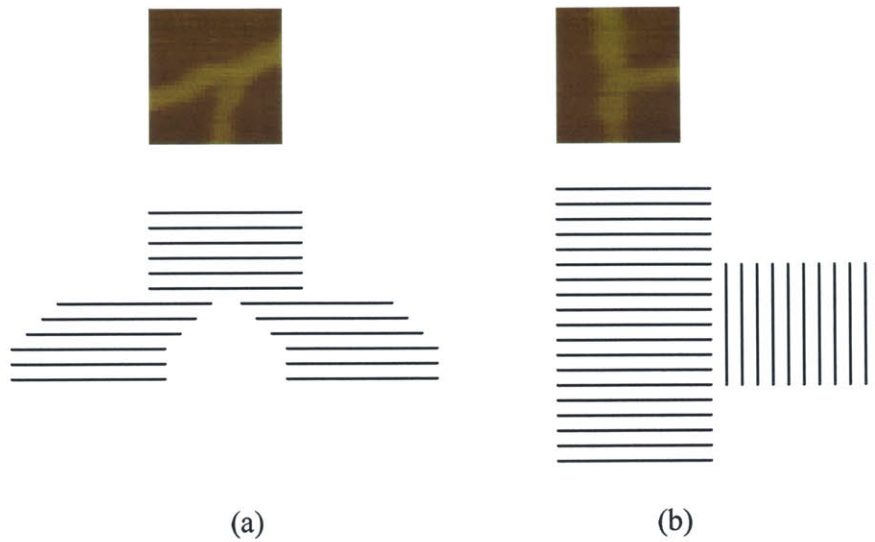


Figure 19. Branching

5. Conclusion

From based on the AFM images, as the amount of b-peptide increases, self-assembly effect goes down while there are more aggregates forming from binding between streptavidin and biotins. Streptavidin attaches not only biotinylated peptide, but also regular peptide. Streptavidin and biotin can cross-link peptide fibers.

These AFM images can explain the rheometer results for the increased modulus. Dynamic light scattering shows that peptide fiber grows with time because the average fiber length that calculated from the decreasing diffusion coefficients increases with time.

6. Recommendation for future work

First, rheometry experiment is necessary. Even though Gina Kim's data can show the increased modulus due to cross-linking, she used a different protocol that

does not include filtering. Also, her concentration is different. Therefore, modulus measurement is needed for each different protocol.

In order to relate the modulus to cross-linking density, modeling can be needed. As the cross-linking density increases, the modulus increases too. One reason is that strong binding of streptavidin and biotin resists the deformation of gel and another that the persistence length of peptide fiber increases. The modeling is recommended to include these components.

Finally through this research it is desirable that we can understand the factors that affect gel's modulus and also control the factors.

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