

Self-Assembly of Protein Fibrils in Microgravity

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ABSTRACT

Deposits of insoluble protein fibrils in human tissue are associated with amyloidosis and neurodegenerative diseases. Different proteins are involved in each disease; all are soluble in their native conformation *in vivo*, but by molecular self-assembly, they all form insoluble protein fibril deposits with a similar cross β -sheet structure. This paper reports the results of an experiment in molecular self-assembly carried out in microgravity on the International Space Station (ISS). The Self-Assembly in Biology and the Origin of Life (SABOL) experiment was designed to study the growth of lysozyme fibrils in microgravity. Lysozyme is a model protein that has been shown to replicate the aggregation

processes of other amyloid proteins. Here the design and performance of the experimental hardware is described in detail. The flight experiment was carried to the ISS in the Dragon capsule of the SpaceX CRS-5 mission and returned to Earth after 32 days. The lysozyme fibrils formed in microgravity aboard the ISS show a distinctly different morphology compared to fibrils formed in the ground-control (G-C) experiment. The fibrils formed in microgravity are shorter, straighter, and thicker than those formed in the laboratory G-C experiment. For two incubation periods, (2) about 8.5 days and (3) about 14.5 days, the average ISS and G-C fibril diameters are respectively:

Period 2 $D_{ISS} = 7.5\text{nm} \pm 31\%$,
and $D_{G-C} = 3.4\text{nm} \pm 31\%$

Period 3 $D_{ISS} = 6.2\text{nm} \pm 33\%$,
and $D_{G-C} = 3.6\text{nm} \pm 33\%$.

Key words: Microgravity; Self-Assembly; Protein; Protein Fibrils; Protein Fibril Morphology; Atomic Force Microscopy; Neurodegenerative Diseases; NanoLab; International Space Station

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INTRODUCTION

Deposits of insoluble protein fibrils are known to be associated with amyloidosis and neurodegenerative diseases such as Alzheimer's,

Dementia, and Parkinson's (Ross and Poirier, 2004; Lansbury and Lashuel, 2006; Kelly, 1996). Several different proteins are involved in the different diseases. All of these so-called amyloid proteins are soluble in their native conformation *in vivo*, but over time, they all form insoluble protein fibril deposits with a similar cross β -sheet structure (Sunde and Blake, 1997; Jimenez et al., 2002).

Amyloid proteins will also form protein fibril deposits *in vitro* with the same cross β -sheet structure as *in vivo*. They self-assemble into fibrils directly from solution when the conditions, such as protein concentration, pH, ion concentration, and temperature are right. Depending on these parameters, different pathways to fibril formation may be followed. Protein monomers either assemble directly into fibrils or follow an intermediate aggregation of oligomers that form before the formation of fibrils (Aggeli and Boden, 2006; Estroff and Hamilton, 2006; Perutz et al., 2002; Hill et al., 2011; Necula et al., 2007; Pellarin and Caflisch, 2006). These different pathways affect fibril diameter, length, and helicity (Woodard et al., 2014; Ward et al., 2012; Dahlgren et al., 2002; Kaye et al., 2003). The study of amyloid fibril formation *in vitro* helps to inform amyloid disease research.

Amyloid formation is not limited to disease-associated proteins, but also occurs in proteins not associated with any known amyloid diseases (Guijarro et al., 1998; Chiti et al., 1999; Kallberg et al., 2001). Hen egg white lysozyme (HEWL) is a prime example of a non-disease associated protein in which amyloid fibrils can form. It shares a very similar morphology with amyloid fibrils formed from disease-associated proteins (Bucciantini et al., 2002). Human mutants of lysozyme are an example of a protein related to organ-specific forms of amyloidosis (Pepys et al., 1993; Canet et al., 1999). These disease-related mutants are also morphologically similar to native lysozyme (Hill et al., 2011). Amyloid disease-related proteins, which are much more difficult to acquire, will form fibrils under normal physiological conditions ($T \sim 37^\circ\text{C}$) whereas HEWL requires $T \sim 50^\circ\text{C}$. The reason for this is that the proteins must be near their denaturing temperature for fibril formation to occur. The growth of lysozyme fibrils has been studied extensively in the laboratory; it provides a model for the study of this self-assembly process (Booth et al., 1997; Frare et al., 2006). Figure 1 shows atomic force microscope (AFM) images of early and late stages of the growth of fibrils from a solution of lysozyme proteins.

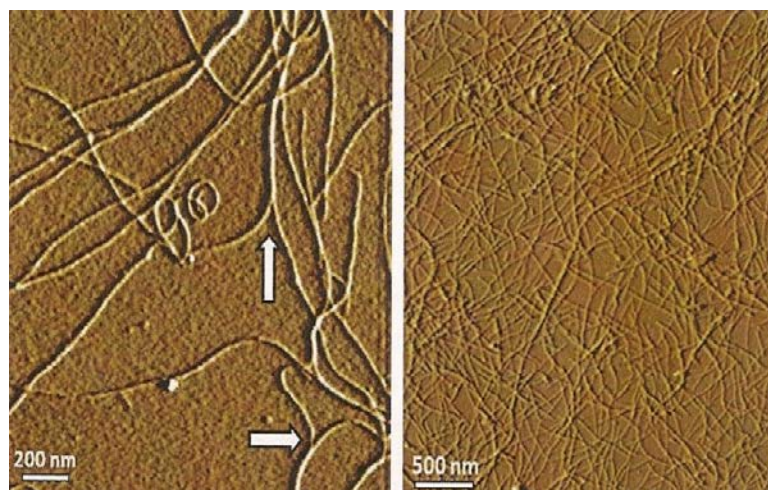


Figure 1. AFM images of lysozyme aggregation showing advanced stages of the amyloid fibril formation process (Woodard et al., 2014). Left: the merging of fibrils into a helix configuration is indicated by the two arrows. Right: A tangled interlocking network prevents fibrils from rotating and halts helix formation. (Note the different image scales.)

To date, most protein aggregation studies have been carried out in ground-based laboratories. After fibril formation and continued incubation these fibrils interact and form macromolecular structures with mature fibrils interweaving with each other (see Figure 1). This network of fibrils can suspend the solution and convert the surrounding fluid into a gel with increased viscosity and opacity (Terech, 2006). These networks create the building blocks for protein plaques to form (Kodali and Wetzel, 2007; Chiti and Dobson, 2006). Currently the morphology of fibril growth cannot be predicted (Bitan et al., 2005; Kowalewski and Holtzman, 1999).

Understanding the colloidal chemistry and biochemistry of amyloid fibril formation is helped by controlling process parameters, such as manipulation of the solution's stabilization with ions, changes in temperature, or pH. It may be possible to inhibit or accelerate amyloid fibril formation. For example, increased salt concentration in the initial buffer solution can increase aggregation and gelation rates (Woodard et al., 2014; Hill et al., 2009, 2011; Fujiwara et al., 2003; Wang et al., 1996).

Gravity has an effect on biological processes even at the cellular level (Tabony et al., 2007). Self-assembly and self-organization of molecules into larger macromolecular structures can be affected by the presence of this weak directional external force. In a space-based laboratory gravitational pressure gradients in fluids do not develop, so natural buoyant convection is not present. Microgravity is a realistic environment to emulate cellular conditions, where surface tension effects dominate viscous effects. The fibril growth process must rely on the movement of protein molecules onto and off of the growing fibrils. In microgravity, surface tension and molecular diffusion will be the dominant interactions.

Although life probably arose in a planetary environment, molecular self-assembly is also of interest to the study of the origin of life. The development of cellular activity was dependent upon the creation of large complex molecular structures in the chemical and environmental

conditions present when life originated. Experiments carried out in microgravity may therefore lead to a better understanding of the dynamics driving cellular molecular self-assembly process. Florida Tech's Self-Assembly in Biology and the Origin of Life (SABOL) study demonstrated protein fibril growth in microgravity onboard the International Space Station (ISS) using a novel, autonomous NanoLab. Protein fibrils grown in microgravity demonstrate a morphology significantly different from samples grown on Earth in a nearly identical Ground-Control (G-C) system.

MATERIALS AND METHODS

Buffer/Lysozyme Solution

The buffer/lysozyme solution for the SABOL experiment was chosen to optimize the fibril growth period from onset through gelation of 20 to 30 days, the estimated time in microgravity. The optimal methods for lysozyme preparation, fibril formation, and fibril characterization are well understood (Burnett et al., 2014; Hill et al., 2011; Woodard et al., 2014). The buffer solution was prepared with 10 mM glycine in DI water, titrated to pH 2.5 with HCl. To ensure uniformity across samples, the titration was performed in a large beaker of solution prior to the addition of lysozyme. The final HCl concentration was approximately 36 mM. Lysozyme (BSG, Napa, CA) was then dissolved in the buffer solution at a concentration of 20 mg/mL. Acidic conditions are required and at this pH the lysozyme has a charge of approximately +26. Heating the sample close to the denaturing temperature of 55°C (Hill et al., 2011) is also required. At this temperature lysozyme begins to unfold from its native conformation and increased Brownian motion assists the Van der Waals attractive forces in overcoming the electric repulsive forces leading to aggregation.

Atomic Force Microscope

The samples were imaged with a Molecular Imaging multi-purpose scanner Atomic Force

Microscope (AFM). PicoView version 1.12 AFM software was used, and all images were acquired in contact mode. A Bruker SNL-10 silicon-tip on nitride cantilever was used, and an Olympus IX71 optical microscope was used to center the laser light reflecting off the cantilever. The quadrant photodiode's position was then adjusted to null the signal. Cantilever probes are typically 600 nm in height with a 10 nm radius of curvature and a force constant of 0.12 N/m. AFM tip with curvature radii greater than 30 nm or damaged were replaced.

A voltage set-point of 3 volts was used, translating to 5.4 nN of load force due to an average of 15 mV/nm deflection sensitivity and 0.12 N/m cantilever elasticity. This set-point was used for the majority of images. Raster speed ranged from 1 to 4 lines/sec, and all of images are 1024 x 1024 pixels. Fields of view (FOV) of 2x2, 5x5, 10x10, and 20x20 μm were the most common. Contact mode, where the probe moves up and down to maintain the constant set point force, was used throughout. Topographic images were used both to determine three-dimensional structure and for dimensional measurements, while deflection images were used to demonstrate shape and texture.

Lysozyme Fibril Deformation

Lysozyme fibrils deform under the force applied by the cantilever. The cantilever load force is determined by the force set-point. A force set-point of 3 volts was used for all AFM images in the SABOL experiment. A calibration was determined by measuring ground-based lysozyme fibril heights for increasing values of the force set-point. Images were taken with the least amount of force first, then increasing until the samples no longer produced readable data. Figure 2 shows height vs. force set-point data for lysozyme fibrils. Each data point is an average of 20 lysozyme fibril height measurements from each force set-point image. The error bars shown are the measurement standard deviations.

Fibril height decreases with increasing load force. A linear fit to this trend, shown by the dashed line, has an R-squared value of 0.93 and a y-intercept of 2.29 nm. All topographic images in this study used a force set-point of 3 volts. Figure 2 can thus be used to correct height measurements of lysozyme fibrils to heights with no load force applied:

$$h = h_m * 1.294$$

where h_m is the measured height, 1.294 is the calibration constant, and h is the undeformed height.

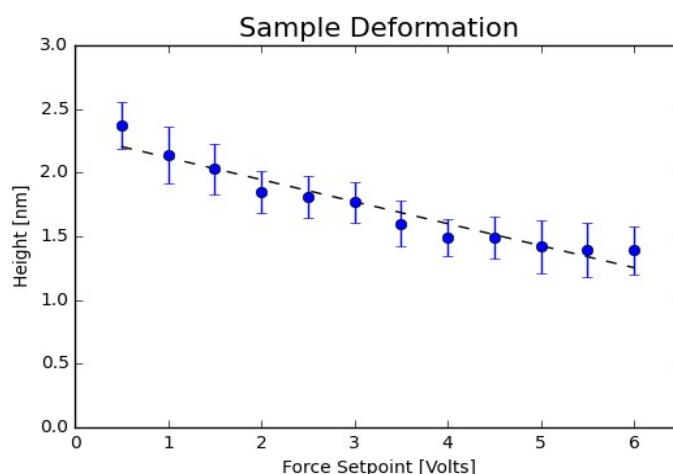


Figure 2. Deformation of lysozyme samples under different load forces (force set-points). Each data point is an average of 20 lysozyme fibril height measurements made at each force set-point. Error bars are the measurement standard deviations. The dashed line is a linear fit to the data.

Sample Preparation

Samples of the aggregated protein were prepared for AFM imaging as follows: First, both undiluted and diluted slides were prepared to ensure protein fibrils were visible against the flat mica substrate and not covered with excess solution protein. Samples were preserved at 4°C, since freezing causes damage to the fibrils.

Drawing lysozyme gels into a standard pipette resulted in fibril breakage due to the gel's large viscosity resulting in fluid shear as the gel was drawn through the small orifice of the pipette (Woodard *et al.*, 2014). Consequently, when pipetting fibrils and/or gels, the tips of the pipettes were first cut off to enlarge the orifice diameter to ~1 mm.

To prepare AFM slides, 10 µL of 0.01 N NaOH was applied near the center of a freshly peeled mica slide to precharge the substrate. After two minutes, 10 µL of sample was applied to the substrate at the same location. After an additional 10 minutes, the sample was rinsed with 2 mL of DI water by allowing the water to flow over the substrate to remove salts and unbound proteins. The water was drained and any droplets adhering to the substrate were removed with a tangentially applied soft jet of nitrogen gas. The slide was then dried in an oven set to 55°C for 2 hours.

SABOL Hardware for the International Space Station Experiment

The Self Assembly in Biology and the Origin of Life (SABOL) experiment was designed to study the self-assembly of lysozyme fibrils in microgravity. An exploded view of the hardware is shown in Figure 3. The experiment is housed in a 1U NanoRacks chassis, measuring 10x10x15 cm. There are 9 vials arranged in a 3x3 array. Each vial has linear stepper motor actuation mechanism, to introduce protein powder into the buffer solution, an individual heater and thermal control system. There is a custom aluminum shell, a support structure for the vials, and a USB connector for power. There are two printed circuit boards (PCB). The side PCB is used to measure and control the temperature of each vial individually and perform data acquisition. The top PCB contains the components needed to operate the actuation mechanisms at the appropriate times. Time is based on an internal battery powered timer to protect against ISS main power loss events.

The fully assembled SABOL hardware with the outer cover removed is shown in Figure 4. All 9 linear stepper motors can be seen above the vial support structure and 3 of the insulated vials can be seen in Figure 4.

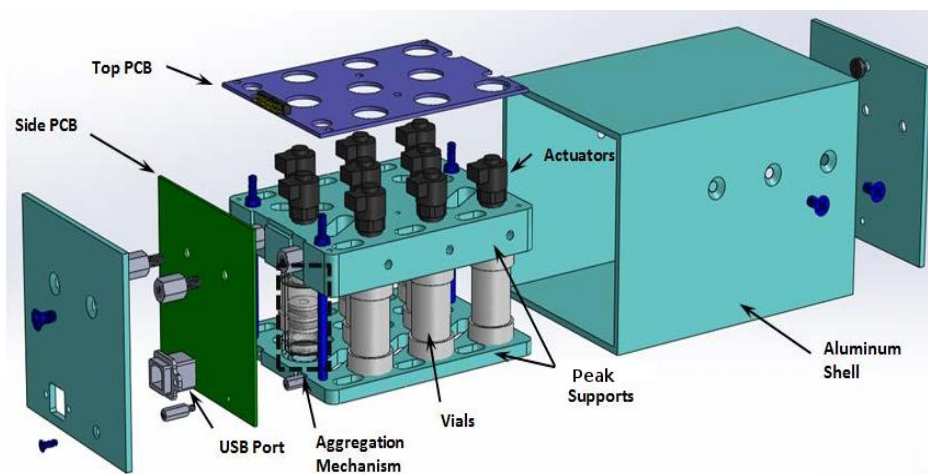


Figure 3. An exploded view of the SABOL experiment. The hardware was designed to fit within the volume, mass, and power constraints of a 1U NanoLab module. There are 9 independently operated vials used to provide a range of incubation times covering the growth phase of lysozyme fibrils.



Figure 4. Fully assembled SABOL NanoLab with samples loaded. Unit shown just before the outer shell was installed.

Actuation mechanism

An important design element of the SABOL experiment was to ensure that the lysozyme powder remained separated from the buffer solution until the incubation period in orbit is initiated. Furthermore, the vials must maintain a seal at all times and not allow air bubbles to form. The vials and all of their internal components are made out of Polypropylene. Two views of a vial are shown in cross-section in Figure 5. The image on the left shows a vial in the unactuated configuration; the image on the right shows it in the actuated configuration. Each vial has two separate compartments initially isolated from each other. One compartment, containing the buffer solution, consists of a 22 mm diameter tube with a volume of 2.5 mL, bounded on the bottom by the floating piston and on the top by the loading piston. The other compartment consists of the filling slot on the side of the loading piston, with just enough volume to hold 0.05 g of lysozyme powder. The loading piston has a threaded hole in its top that is attached to the shaft of a linear stepper motor. Actuation occurs when the stepper motor drives the loading piston down, uncovering the filling slot and bringing the lysozyme powder

into contact with the buffer. The floating piston reacts back to maintain a constant volume.

Thermal control system

Each vial has an independent thermal control system consisting of Nicrome heater wire wrapped around each vial, a layer of insulation over the heater wire, and a thermocouple bonded into a capped hole in the bottom of the floating piston. The control system consists of a simple set-point regulation system $T = T_o \pm \Delta T$ originally chosen as $T_o = 55^\circ\text{C}$ and $\Delta T = 1.5^\circ\text{C}$. The 5 Watts of electrical power available to the 1U module through its USB connector was only sufficient to run 4 of the heaters at once, so a heating schedule was developed where no more than 4 heaters were on at a time. This allowed incubation times to cover from 3 to 27 days in increments of 3 days. One vial remained unheated to serve as a control.

The start of an incubation period for a given vial is implemented as follows: first all heaters are turned off to provide enough power to run a stepper motor. The stepper motor of the vial to be actuated is turned on, run to completion, and then turned off. That vial's heater is then turned on and heaters for the other vials still within their incubation periods are turned back on. When the

time is reached for a vial's incubation period to end, its heater is turned off. Since the heating and cooling time constants for the vials are nearly an hour, and the incubation time periods are days, the few minutes it takes to execute the vial actuation

has negligible effect on the incubation. The incubation time period for a given vial is determined as the time the temperature exceeds 50°C.

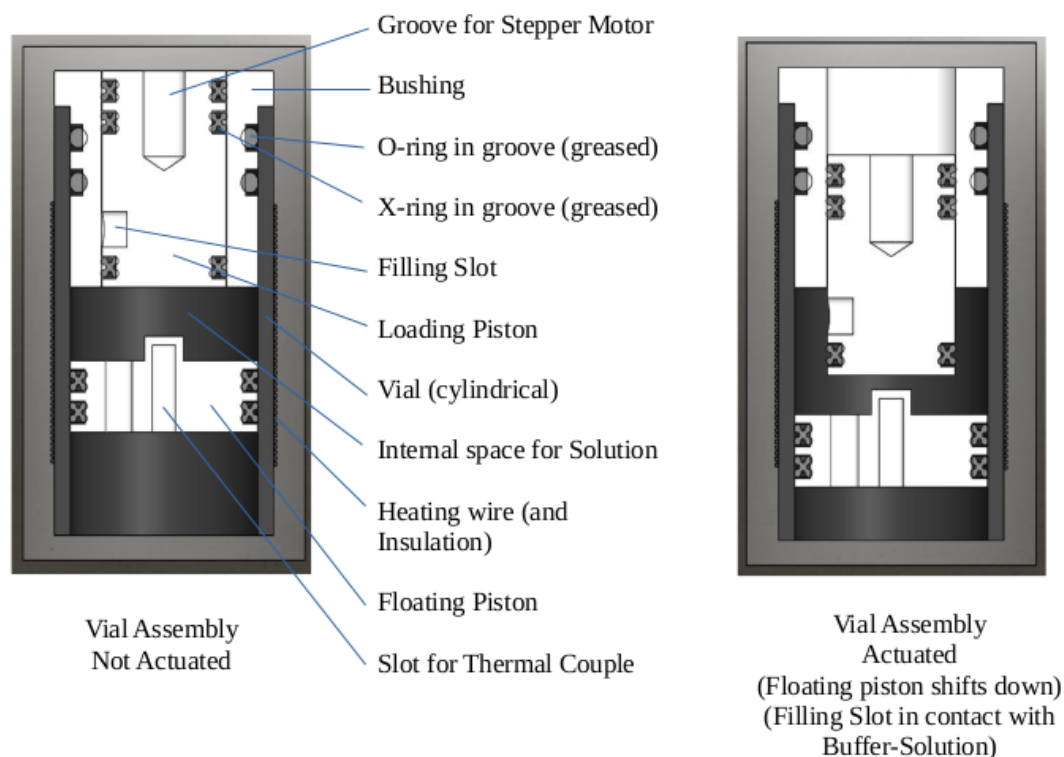


Figure 5. Cross-section of polypropylene vials before and after actuation.

Ground-Control NanoLab

An identical Ground-Control (G-C) NanoLab was built so that it could be run on ground with the same timing protocols and environmental conditions as the ISS NanoLab, except for the effect of microgravity.

Preparation and flight

The day before the experiment was handed over to NanoRacks, samples were loaded as follows: With the bushing and loading piston removed, each vial was filled with 2.5 mL of buffer solution and the floating piston was pushed up, bringing the fluid level up to the top of the vial. Then dry lysozyme protein powder was

placed in the open slot of the loading piston. The loading piston was placed inside the bushing, sealing off the protein powder. The bushing with the loading piston installed was then placed at the top of the vial and inserted in a manner that sealed the buffer solution with no air bubbles. The complete vial assembly was then threaded onto the stepper motor shaft. This was repeated for each of the nine vials.

The SABOL experiment was carried to the International Space Station (ISS) in the Dragon capsule of the SpaceX CRS-5 mission and returned to Earth in the same Dragon capsule after 32 days. Table 1 gives the duration times for both the ISS and G-C NanoLab's. The G-C NanoLab was run for the same amount of time as the ISS NanoLab. No telemetry was available from either

to monitor their progress. The ISS NanoLab was kept near 4°C from deorbit throughout

splashdown, retrieval, and transportation back to Florida Tech.

Table 1. NanoLab Timeline (EST).

	Plugged In	Duration
ISS NanoLab	Jan. 13th 1:22 PM	25d 18h 28m
G-C NanoLab	Feb. 10th 4:41 PM	25d 18h 28m

ISS NanoLab post-flight handling

The ISS NanoLab memory chip, containing temperature data, was accessible after removal of the experiment's outer shell. Figure 6 shows temperature as a function of time for all 9 vials. Vial 1 was a control that was not heated. The unheated or ambient temperature within the NanoLab, as indicated by vial 1, remained between 33°C and 35°C for the duration of the experiment. Previous testing showed that fibril growth would not start until the temperature was greater than 40°C. Vials 2 through 9 show proper heating for different amounts of time. As one vial turned off another vial actuated the protein into the buffer solution. Then that vial was heated to the incubation temperature.

Next, the four bolts holding the top and bottom support structure together were removed. All 9 vials were unscrewed from their stepper motors, being careful not to disturb the position of the loading pistons. Each of the nine vials were placed in individual plastic bags labeled with the vial's position and stored in a 4°C refrigerator. The top support could then be examined to see if the stepper motors had fully actuated their respective loading pistons. Figure 7 shows that only 3 of the stepper motor shafts had extended, so only 3 of the vials had successfully actuated while in orbit aboard the ISS. They were vials 5, 6, and 8.

The vial 5 heater was turned on at the beginning of the experiment and turned off at day 15. The temperature remained at about 53°C for the duration of its operation. It dropped to 37°C when turned off and decayed to 36°C by the end of the experiment. Vial 8 heater turned on at day 18. The temperature remained at about 51°C until

the experiment was terminated at day 25.75. The vial 6 heater was turned on at day 24. It remained at 51°C until the experiment was terminated at day 25.75. Thus, these three vials provide samples with incubation times (Vial: Time) as follows: (5: 15 days), (8: 7.75 days), (6: 1.75 days).

The loading pistons were extracted from each vial, samples were collected with a 200 µL pipette, and samples were prepared for imaging. The pre-extraction positions of the loading pistons confirmed that only vials 5, 6, and 8 had actuated properly. Also, it was noted that the loading piston slots of vials 5 and 8 had solution remaining in them, and a smooth layer of fluid flush with the outside of the slot. The solution within these slots appeared more viscous than the majority of the solution inside the vial. A sample of solution from the loading piston slot was taken for vials 5 and 8.

G-C NanoLab handling

The Ground-Control experiment had some unintended differences from the ISS experiment. The G-C NanoLab contained only 8 vials, as one was damaged during testing. To compensate for this, a thermocouple was suspended in the position of vial 1 to measure ambient air temperature. After completion of assembly and sample loading, the G-C NanoLab was plugged into a USB converter of an uninterrupted power supply (UPS) at Florida Tech. The G-C NanoLab was powered on for the same length of time as the ISS NanoLab, as shown in Table 1. The G-C NanoLab was then cooled to 4°C and kept at that temperature for 6 days to match the conditions of the ISS NanoLab. The G-C NanoLab was then prepared for imaging in the same way as the ISS

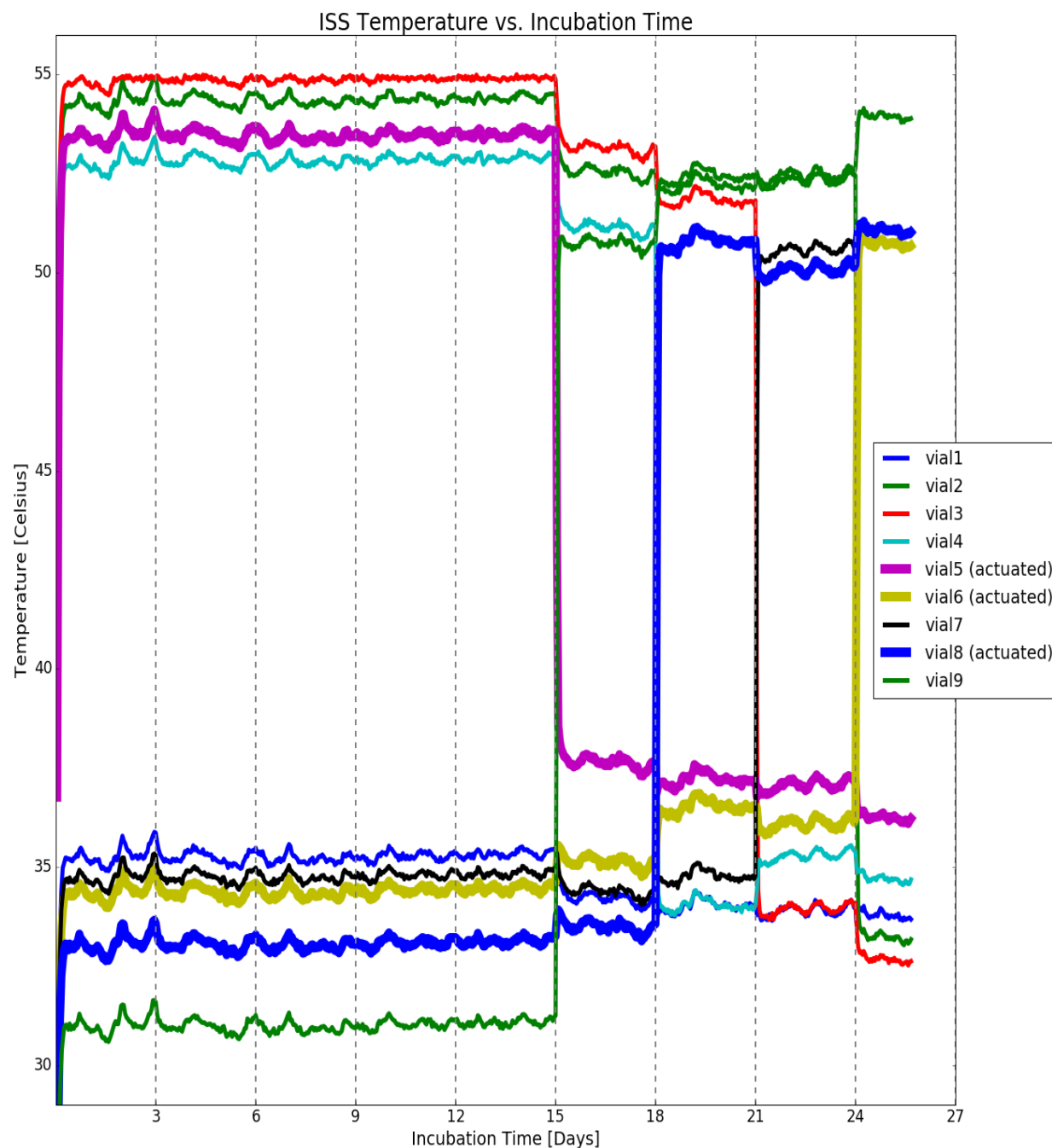


Figure 6. ISS Vial Temperature versus Time. Temperature profiles for each of the ISS NanoLab vials show the time when each heater was turned on, raising the temperature to within the aggregation range (Hill et al., 2009; Woodard et al., 2014), held there for its incubation period, and then turned off.

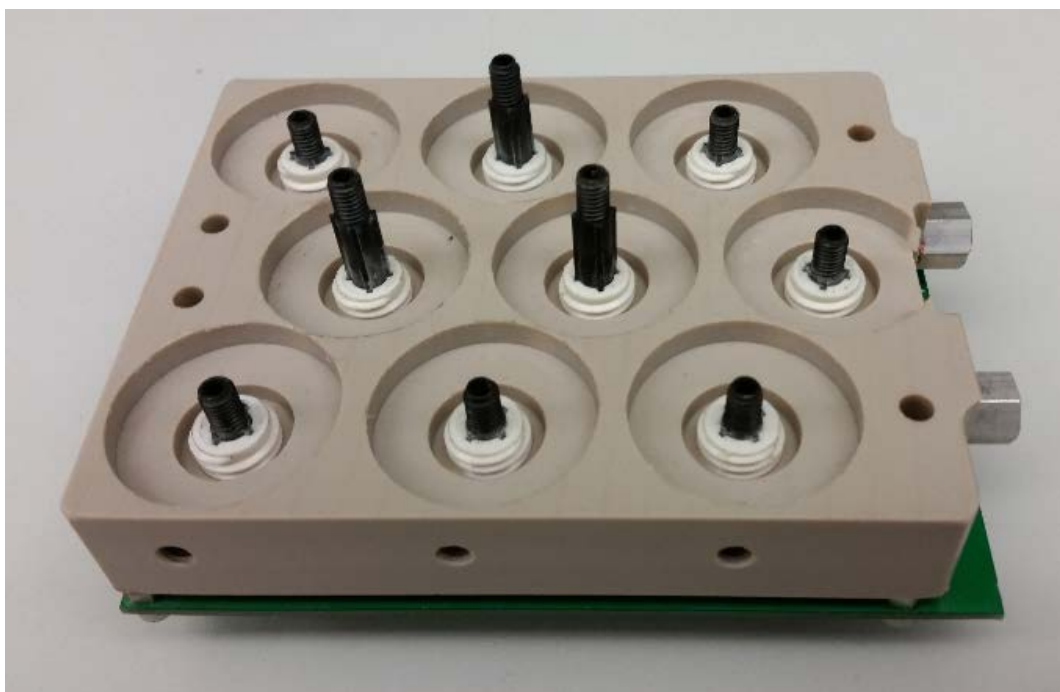


Figure 7. Top support plate that holds the vials in place. The stepper motor shafts stick through this support, clearly showing that only vial positions 5, 6, and 8 were actuated.

NanoLab. There was no viscous solution in the loading piston slots as seen in the ISS vials.

Figure 8 shows temperature as a function of time for all vials of the G-C NanoLab. The actuation and heating times were accidentally reversed relative to the ISS NanoLab; the shorter incubation times were first on the G-C NanoLab, whereas they were last for the ISS NanoLab. When the experiment was stopped 1.25 days before the planned stop at day 27, this had the unintended effect of losing 1.25 days from different incubation intervals of the ISS and G-C data.

Most of the vials in the G-C NanoLab, except vial 7, reached the incubation temperature. The ambient air temperature inside the G-C NanoLab was between 30°C and 34°C. For comparison with the ISS NanoLab, the following G-C NanoLab vials with the similar incubation times can be used — (9: 13.75 days), (4: 9 days), (2: 3 days). The

time scale for changes in fibril growth is a few days so this difference between ISS and G-C samples is still useful.

RESULTS AND DISCUSSION

Slides for AFM imaging were prepared, as discussed earlier, using solution taken from the three ISS NanoLab vials that actuated properly: vials 5, 6, and 8. Figure 9 shows an AFM image of solution taken from ISS NanoLab vial 8. This sample was incubated in microgravity for 7.75 days at 51°C. The image was created with a 5 x 5 µm FOV. There is a clear difference between the lysozyme fibrils seen here and those routinely seen in samples formed in ground-based laboratories. In Figure 1, for example, fibrils formed in microgravity on the ISS are shorter, straighter, and thicker (larger diameter) than samples formed in the laboratory.

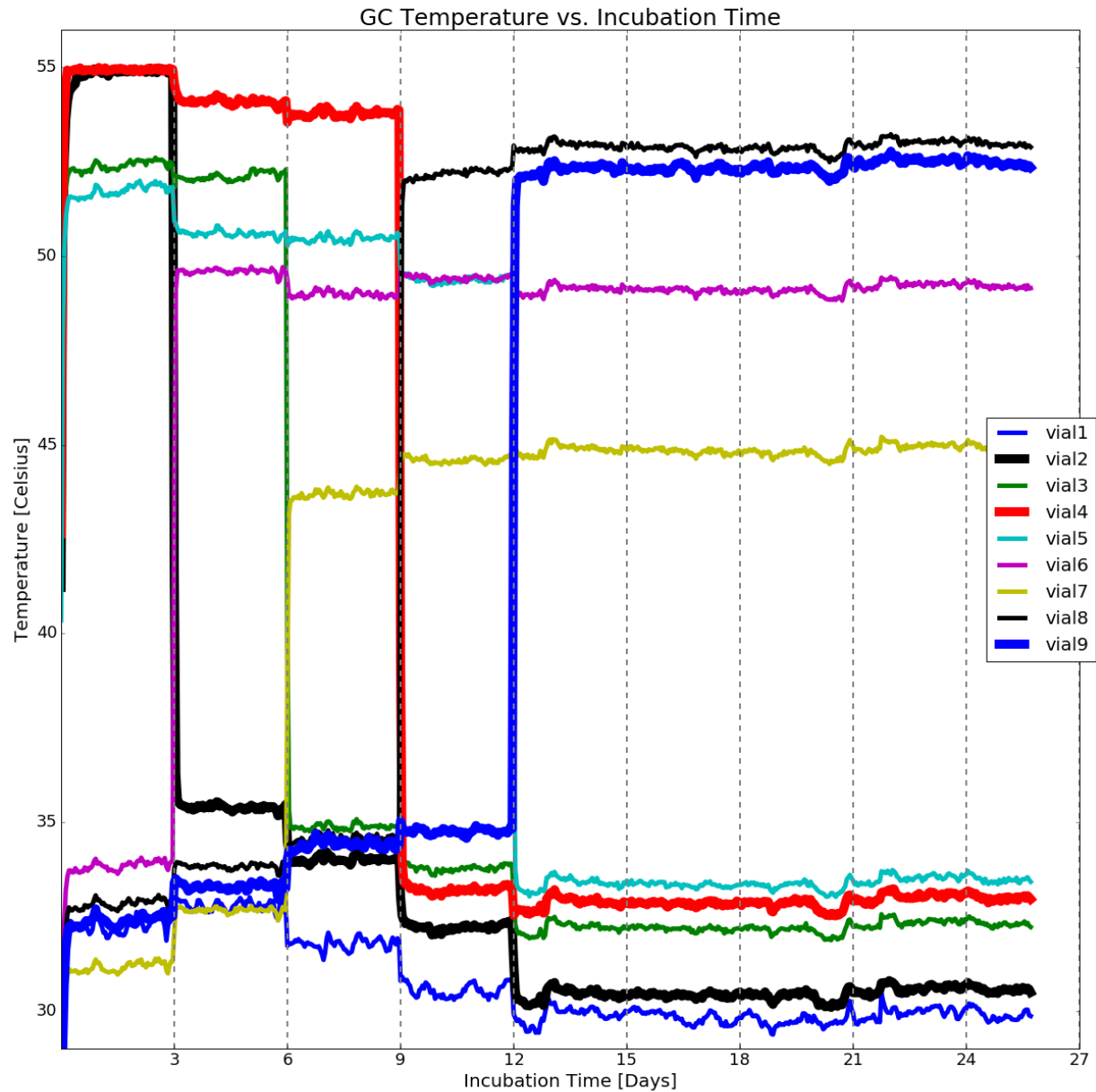


Figure 8. Temperature profile of each vial within the G-C NanoLab. The graph shows the time when each heater was turned on, raising the temperature to within the aggregation range (Hill et al., 2009; Woodard et al., 2014). The bold lines represent the temperature profiles from the vials that incubated for periods of time close to the 3 vials that fully actuated in the ISS NanoLab.



Figure 9. AFM image of solution from ISS vial 8, 7.75 days of incubation, 5 x 5 μm FOV.

Fibrils formed in ground-based laboratories normally use a significantly different vial shape and protein powder mixing system. To determine if the change in fibril morphology could be attributed to the more complex process of actuation or the significantly different geometry of the ISS and G-C NanoLab vials, a complete set of slides for AFM imaging were prepared using solution taken from the three G-C NanoLab vials with closely matching incubation times: vials 2, 4, and 9. Although not ideal, the difference in incubation times of 1.25 days is still a useful comparison since the time scale for significant change in fibril growth is on the order of days. Also, in microgravity, fluid motion by surface tension is likely to play a large role so the comparison of fibril growth in identical vials is important. Remember the more viscous fluid present in two of the loading slots of the ISS NanoLab.

Another difference between the ISS and G-C NanoLabs is the external forces due to launch, re-entry, and landing experienced only by the ISS

NanoLab. During launch the buffer solution and protein powder were in separate chambers so this should have no effect on fibril growth. Also the re-entry and splashdown forces should have no effect since already grown fibrils are stable. Furthermore, the ISS NanoLab was in a transfer bag that included bubble wrap to dampen vibration and the temperature of the ISS NanoLab was held well below the aggregation temperature.

Figure 10 shows representative images from each of the three incubation groups. The images on the left are of ISS formed fibrils, those on the right are of G-C formed fibrils. The incubation time in days is shown for each image. This set of images shows a clear morphological difference between fibrils formed in microgravity and those formed in the ground-control unit. For the first group, the ISS images show no fibril formation but numerous small isolated structures while the G-C images show fully formed fibrils already longer than the 5 μm FOV. For the second incubation group, the ISS fibrils have formed but they are relatively straight and short (significantly

less than the 5 μm FOV), whereas the G-C fibrils are fully formed, mature fibrils. For the third incubation group, the ISS fibrils show continued growth with some fibrils almost as long as the 5

μm FOV and maybe a few more complex structures forming; the G-C fibrils show continued formation of long thin complex structures.

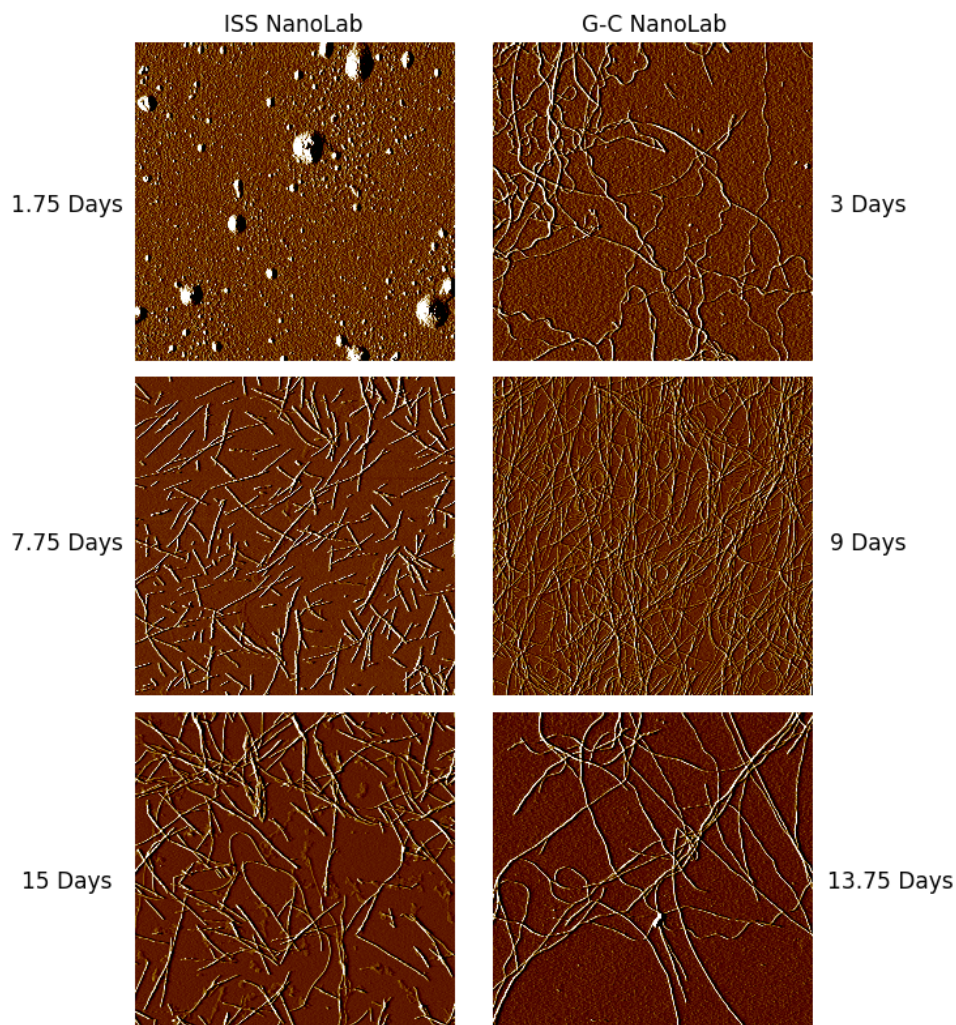


Figure 10. Lysozyme fibrils formed in microgravity on the ISS versus lysozyme fibrils formed in the G-C unit under the effects of gravity. All images have a 5 μm FOV.

One difference in the fibril structure present in these images is that the microgravity formed fibrils consistently appear about twice as thick as the G-C formed fibrils. To quantify this difference, the height and width of many fibrils was measured for several images from each ISS and G-C incubation groups. Using the PicoView v1.12 software, a trace was made perpendicular to every accessible fibril at 3 separate places on each fibril. The height and width were determined from

the trace. All together there were 18 ISS images used, giving 513 fibril measurements, and 14 G-C images used, giving 232 fibril measurements.

The height measurements can be directly related to fibril diameter using the deformation calibration determined earlier. The width measurements are less reliable since the radius of curvature of the AFM tip is approximately 10 nm, considerably larger than the width of the fibrils.

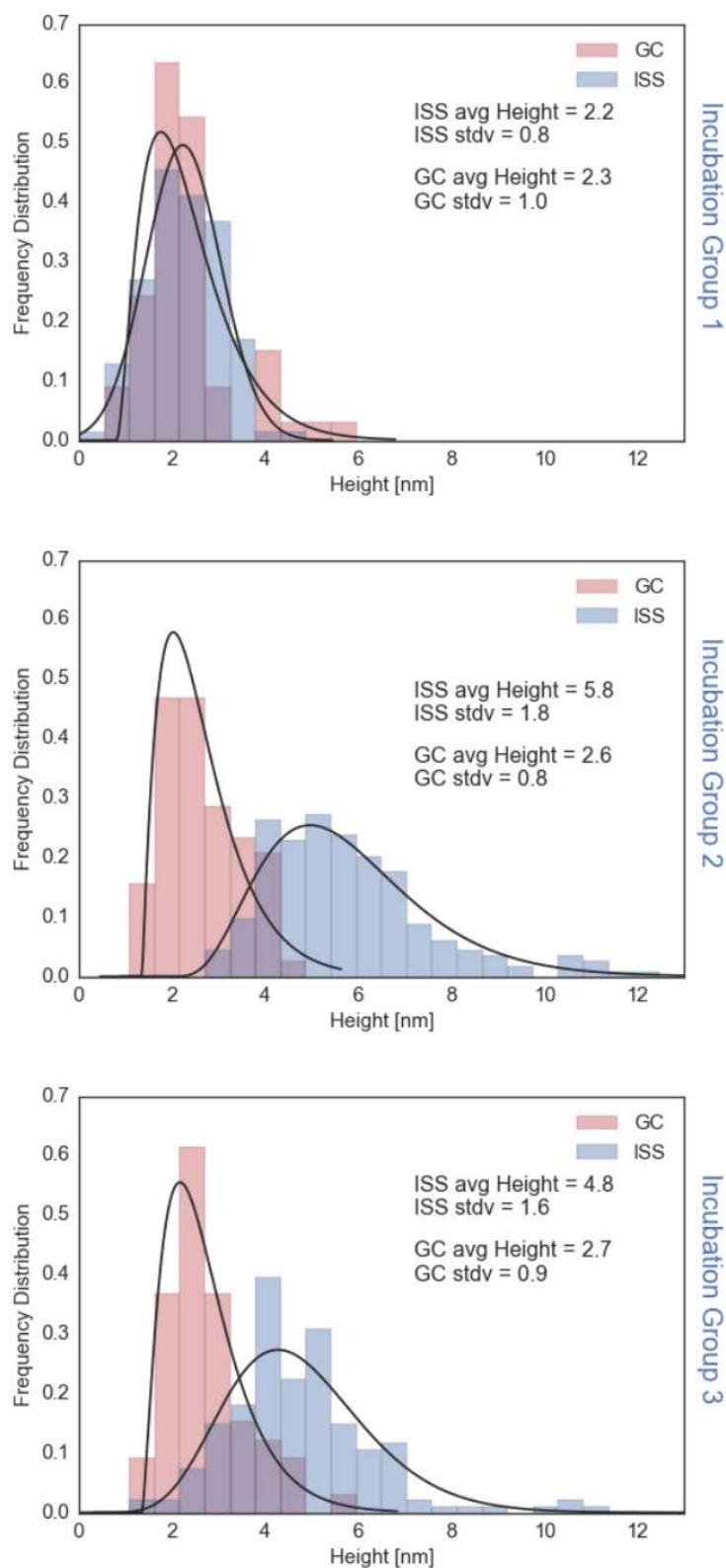


Figure 11. Heights of protein fibrils formed in microgravity (ISS) compared to protein fibrils formed in a Ground-Control (G-C) experiment.

Figure 11 shows a frequency distribution plot of fibril heights for each of the three incubation periods. The upper figure is for the first incubation period. The average heights of the ISS and G-C fibrils are $H_{ISS}=2.2\pm0.8$ nm and $H_{G-C}=2.3\pm1.0$ nm, respectively. The average height and standard deviation are calculated directly from the data, not the gamma-fit trend line. At this incubation period the heights are the same within the experimental error. It should be noted however that at this stage the ISS images showed only small isolated structures and no fibrils.

The middle figure is for the second incubation period. The average heights of the ISS and the G-C fibrils are $H_{ISS}=5.8\pm1.8$ nm and $H_{G-C}=2.6\pm0.8$ nm, respectively. This shows that ISS fibrils have about twice the diameter as G-C fibrils.

The lower figure is for the third incubation period. The average heights of the ISS and G-C fibrils are $H_{ISS}=4.8\pm1.6$ nm and $H_{G-C}=2.7\pm0.9$ nm, respectively. Again, the ISS fibrils have about twice the diameter of the G-C fibrils.

Another characteristic of these data is that, for incubation groups 2 and 3, the frequency distribution for the ISS grown fibrils is significantly broader than the G-C grown fibrils. This could indicate an actual physical difference, with the microgravity formed fibril heights being more variable than the G-C formed fibrils, or it could be that the measured errors in fibril heights are proportional to the fibril heights. In the latter case, the fractional error for the ISS and G-C measurements should be the same in each incubation group. The fractional errors determined from the data above are: incubation group 2, $H_{ISS}=5.8$ nm $\pm$ 31% and $H_{G-C}=2.6$ nm $\pm$ 31% and for incubation group 3, $H_{ISS}=4.8$ nm $\pm$ 33% and $H_{G-C}=2.7$ nm $\pm$ 33%. So, it appears that errors in fibril height measurements are proportional to fibril heights. This also indicates that the height-to-diameter calibration determined with 2.7 nm diameter fibrils is applicable to $\sim$ 5 nm fibrils as well. Applying the calibration to these data gives the average fibril diameters as:

Incubation Group 2: $D_{ISS} = 7.5$ nm $\pm$ 31%,
and $D_{G-C} = 3.4$ nm $\pm$ 31%

Incubation Group 3: $D_{ISS} = 6.2$ nm $\pm$ 33%,
and $D_{G-C} = 3.6$ nm $\pm$ 33%.

CONCLUSION

The primary objectives of the SABOL experiment were to grow protein fibrils in microgravity onboard the ISS and to develop the robust capability of conducting protein fibril research on the ISS. Both of these objectives were met. Lysozyme fibrils were grown in microgravity and the results indicate that this is a fruitful environment for the study of this molecular self-assembly process. Two identical sets of hardware were constructed providing a flight unit and a ground-control unit for future research. Both systems performed well. The only major anomaly in the experiment operation was that, for the flight unit, only 3 of the 9 vial actuation mechanisms operated properly. This can be corrected with a small change in the vial design allowing for a linear stepper motor with more force for any future experiments. This hardware can also be modified and used to study other amyloid protein fibrils.

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