IMAGING RIBOSOMES IN FLUID USING THE ATOMIC FORCE MICROSCOPE

by

Hiram Jacob Conley

A senior thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Bachelor of Science

Department of Physics and Astronomy

Brigham Young University

August 2007

BRIGHAM YOUNG UNIVERSITY

DEPARTMENT APPROVAL

of a senior thesis submitted by

Hiram Jacob Conley

This thesis has been reviewed by the research advisor, research coordinator, and department chair and has been found to be satisfactory.

Date	Robert C. Davis, Advisor
Date	Eric Hintz, Research Coordinator
Date	Ross Spencer, Chair

ABSTRACT

IMAGING RIBOSOMES IN FLUID USING THE ATOMIC FORCE MICROSCOPE

Hiram Jacob Conley

Department of Physics and Astronomy

Bachelor of Science

We developed a technique to image ribosomes with the Atomic Force Microscope(AFM) in a fluid environment. We imaged 30S, 50S, and 70S ribosomes in fluid and showed that our data is in agrement with crystallography data. This is an introductory work to enable our group to study ribosomes using the AFM

ACKNOWLEDGMENTS

Thanks to Dr. Davis and Dr. Busath for spending time with me to teach me how to do research and for my wife being fine with me getting home late.

Contents

List of Figures

Chapter 1

Introduction

1.1 Background

Ribosomes are 30 nanometer particle of RiboNucleic Acid (RNA) and proteins. They function as nanoscale factories. The ribosome is responsible for reading the information stored in our genes and assembling amino acids into proteins. Understanding the ribosome is key to understanding the basics of decoding information stored in genes and in designing some future nanotechnologies.

The ribosome is a biological particle. Traditional biological assays have not been able to fully elucidate the ribosome. In order to understand the ribosome, one must understand the chemistry of the bonds that hold the ribosomal subunits together,the physics that determines their motion and the molecular biology that determines their function.

A ribosome can also be thought of as a nanofactory. It is a nanosized particle that reads data from a source and from this data creates something useful. One of the goals of nanotechnology is to consistently and quickly construct useful things on the nanometer scale. The ribosome already does this. It constructs proteins

from amino acids. It does this quickly and with a great deal of accuracy. From a study of ribosomes, one may discover potential solutions to these challenges facing nanotechnology.

The goal of this research is to develop a technique to image ribosomes in a fluid environment that resembles the environment inside a cell. This will enable further research into the dynamics of the ribosome and help understand this nanoscale factory.

1.2 The Ribosome

Ribosome are a mixture of 54 proteins and over 4,000 nucleotides [1]. A ribosome consists of 2 subunits called 50S and 30S in prokaryotes. When the 30S and 50S ribosomes subunits are bound together it is called a 70S ribosome. The main function of the ribosome in a cell is to synthesize new proteins in a process know as translation (see Figure 1.1). A strand of mRNA (messenger RNA) is copied from the DNA in the nucleus and is sent to the cytoplasm. The 30S and 50S subunits of the ribosome attach themselves to the mRNA. Amino acids are connected to tRNAs (transfer RNA) that diffuse inside the cell. There is a specific amino acid bound to each type of tRNA. The ribosome connects a specific tRNA to the mRNA that has a matching code. Then the ribosome grabs the amino acid that is connected to the tRNA. The ribosome then ejects the spent tRNA, ratchets down to the next codon on the mRNA and repeats the cycle. The ribosome strings a specific order of amino acids together to make proteins. A ribosome does this at the fast rate of 10 to 20 amino acids per second. [2] The ribosome also has a very high fidelity rate of about 0.0001 errors per amino acid. [3, 4])

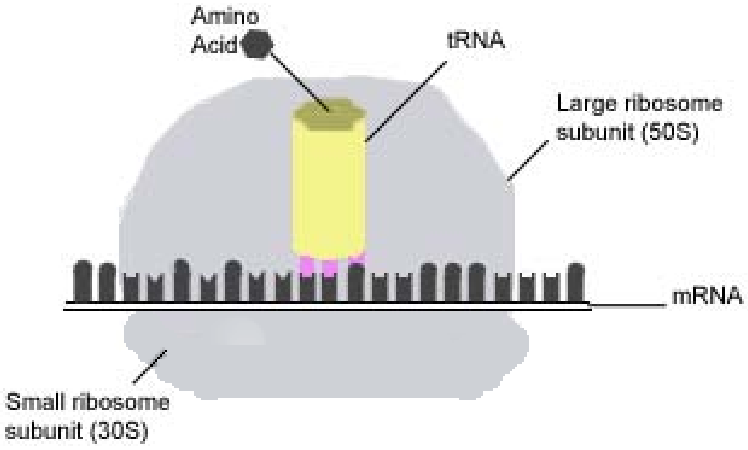

Figure 1.1 A depiction of translation

1.3 A History of Imaging Ribosomes

The ribosome was discovered in 1956 by George Emil Palade. It was thought to be small pieces of mitochondria and was originally named the microsome. Palade used an electron microscope simultaneously with sucrose gradients to determine that they were made of ribonucleic acid and stuck to the endoplasmic reticulum. He renamed the microsome a ribosome and received a Nobel prize for his work. [5]

While people have studied ribosomes since 1956, the molecular processes that govern translation are still not well understood. [6] Crystallographers tried for over twenty years before they obtained a high resolution image of a ribosome subunit. [7] They were able to achieve 2.4 Å resolution for the 50S subunit (Fig, 1.2) and 3.0 Å resolution for the 30S subunit [6, 8](Fig, 1.3). A problem inherent to the use of crystallography is that the crystallization process may cause distortions. [9]

Another method of probing ribosomes is to use cryo-electron microscopy. Ribosomes are frozen and then imaged in an electron microscope. This method is able to achieve 11.5 Å resolution while avoiding the artifacts of crystallography. [9] This technique was used to show that the ribosome undergoes conformational changes. [10]

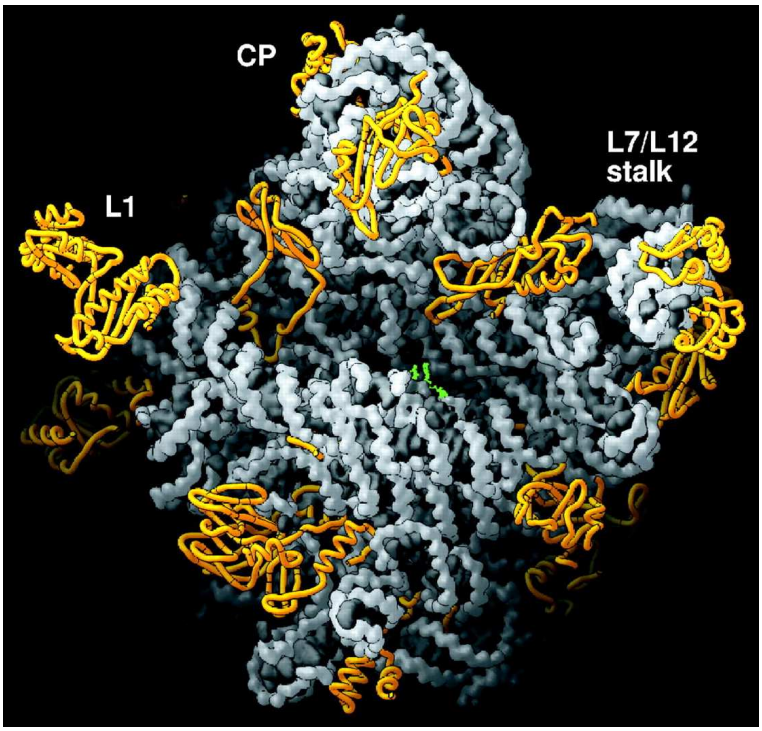

Figure 1.2 2.4 Å resolution crystallography image of the 50S ribosome sub unit. [8]

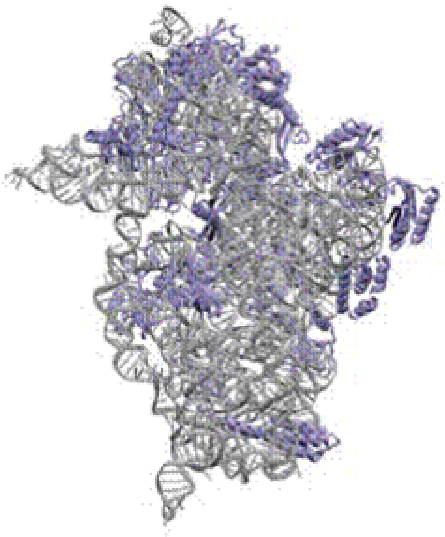

Figure 1.3 3.0 Å resolution crystallography image of 30S ribosome subunits. [6]

Yet cryo-electron microscopy is unable to obtain realtime data on these conformational changes. This technique also requires freezing the ribosome which may affect the ribosome.

Taking another approach, several groups have imaged the ribosome using the Atomic Force Microscope AFM. [11–14] All the groups except for Vanzi et al imaged them on a dry mica surface. Imaging in air dehydrates the ribosomes which may greatly affect their shape. [14] Vanzi et al reported imaging ribosomes with the AFM in fluid, but only published data regarding ribosome density on a mica surface. They also used a low resolution tip with a radius of 20 nanometers. [13].

1.4 Challenges Facing the Ribosome Research Today

All published images of ribosomes require putting a ribosome in a highly unnatural state. Crystallography requires that one crystallize the ribosome. In order to use cryo-electron microscopy the ribosomes needs to be frozen. Most of the published AFM images of the ribosome published were done of dried ribosomes on a mica surface. [11, 12, 14] These environments are not like the environment inside the cell and the ribosomes appear to spread out and shortened. All of these methods require an immobilized ribosome, but the problem is that the ribosome is not a static particle. It is a dynamic particle. A ribosome's function depends on its dynamics. Most forms of microscopy are incapable of probing a dynamic, functioning ribosome.

Although the dynamics of a ribosome is essential to its function, there is a lack of knowledge about the dynamics of the ribosome. Data from cryo-electron microscopy shows the ribosome both in a tight and loose conformations. [15] The underlying conformation changes are considered fundamental to translation, but are not well

understood. [16] Cryo-electron microscopy data has lead to a series of attempts to model ribosomes with computers. [15–17] Some models use normal modes and others assume that there are no normal modes. There is not enough data on individual ribosomes in a dynamic setting to be able to confirm which model is better or to refine the current models to improve them.

Another interesting area of research is to determine all the uses of ribosomes. As a fully functioning nanofactory with amazing speeds and capabilities it should be asked, what information can be gleaned from this system that can be transferred into nanotechnology? Ribosomes need to be explored to see if there are ways that we can have them build electrical and nanocomponents.

1.5 The Atomic Force Microscope

The AFM is a microscope used to image materials on the scale of nanometers. The AFM functions by having a tip (Fig, 1.4) that can be moved along a surface. When the tip bumps into something on the surface, the tip moves. The tip is connected to a cantilever much longer than the length of the tip. A laser is pointed at the end of the cantilever. As the cantilever moves up and down, it causes the reflected laser beam to leave. A photo-diode is used to measure the changes in the reflected position of the laser, and from that information it is possible to generate an image of what is on the surface.

The Pico Scan III in BYU's AFM lab has several modes of usage that make it particularly useful when working with biological samples. The AFM is able to image in what is called taping mode. This is done by sending a sound wave through the sample at special resonance frequencies of the cantilever which causes the tip to move up and down. Lightly tapping the tip along the surface instead of dragging a tip

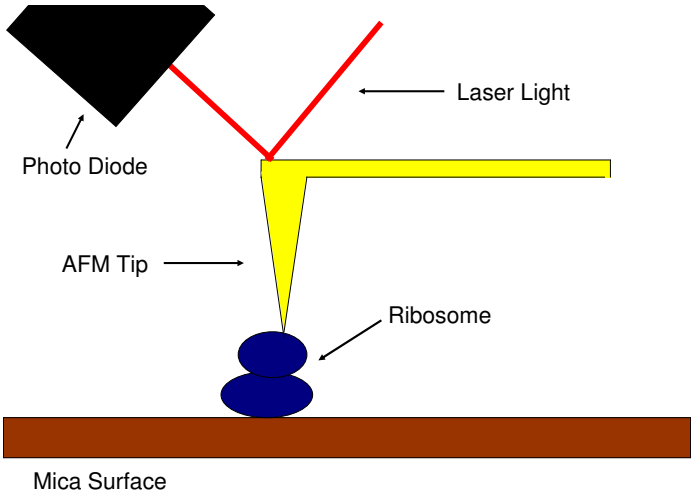

Figure 1.4 Schematic of the AFM

along a surface minimizes the amount of damage to the sample. This is important when imaging biological samples, as they tend to be fragile.

Another main advantages of using the AFM in biological samples is its ability to image in a fluid environment. This enables one to image a molecular process in real time. [18] Allowing one to probe biological processes in a way unavailable with any other technique.

On the downside one is not able to obtain the resolution available with crystallography. The width of the tip limits the resolution one can obtain. Tips with a width of seven nanometers were used. This is the maximum lateral resolution that can be obtained. If a super tip with a width around a nanometer, the maximum lateral resolution is one nanometer. This is slightly better than the resolution of cryo-electron microscopy.

The purpose of this research is not to obtain the highest resolution images of ribosomes. The AFM can not obtain the resolution that crystallography can. The

goal is to add a tool to ribosomal research that will complement the data obtained by other methods. This allows us to be able to study the physics behind the ribosome. The AFM can collect data about ribosome dynamics on individual ribosomes. One can rest a tip on top of a ribosome and measure how much it moves. One can apply forces to a ribosome and see what effects it has on its function. The strength is not in imaging, no mater how impressive, but the other data that the AFM can collect from the ribosome.

1.6 Overview

Using the AFM we were able to image in fluid both 30S and 50S ribosomal subunits as well as the complete 70S ribosome. We obtained very consistent results with 30S and 50S ribosome subunits. We are able to distinguish between the various subunits and the 70S ribosomes.

Chapter two contains an overview on methods in section 2.1, then goes through a detailed explanation of the method. This part of the thesis is about the buffers used, section 2.2, how to prepare the ribosome sample, section 2.3, and the settings of the AFM, section 2.4. This ends with a brief note on imaging the sample, section 2.5. Then the thesis goes on to talk about various problems encountered while trying to image the ribosomes. The effect of RNase is discussed, section 2.6, binding the ribosomes to a mica surface, section 2.7, and the dissociation of ribosomal subunits in solution, section 2.8

Chapter three contains the results of imaging ribosomes in fluid, section 3.1. It also contains a section on analysis of data and how well these results match up with previous research, section 3.2. Finally the thesis concludes with a section 3.3 that suggests further areas of research using the techniques probed in this thesis.

Chapter 2

Methods

2.1 Overview of Methods

The preparation of the samples is critical to obtain consistent images of ribosomes. The main focus of this research was to develop a technique to image ribosomes in fluid. First we prepared appropriate buffers to image the ribosomes. Then we put the ribosomes in the buffers at the appropriate concentrations so that they can be imaged. Next we prepared the AFM to image biological samples in fluid. Then we bound the ribosomes to a mica surface and finally imaged them with the AFM.

2.2 Buffers

There are two main buffers used in this research. The first buffer is the ribosome resuspension buffer. It contains 50 mM $Tris-HCl$ pH 7.5, 150 mM NH_4Cl, 5 mmol $MgCl_2$, and 6 mM BME (C_2H_6S). The other buffer is the tight binding buffer. This buffer contains 50 mM $Tris-HCL$ pH 7.5, 300 mM NH_4Cl, 20 mM $MgCl_2$, and 6 mM BME. The increased amount of $MgCl_2$ helps the subunits of the ribosome bind

together. [19]

2.3 Preparing the Sample

Ribosome samples from E. Coli at 1 mM concentration were obtained from Dr. Jamie Williamson's lab at Scripps. The samples from Scripps were diluted to concentrations more appropriate for imaging with the AFM. The appropriate concentration is about one nM. The solution was separated into 30 μL samples. These small samples are for one-time use. They were flash-frozen in ethanol and dry ice and stored at -80°C for future use.

When it was time to image, a single 30 μL sample was removed from the -80°C freezer and thawed on ice. The sample was then put on a freshly cleaved sheet of mica. It was allowed to sit for a few minutes to allow the ribosomes to bind to the mica surface. About 100 μL of buffer solution was applied to the mica surface to form a droplet of fluid on the surface. The sample was placed in the AFM, and the tip was lowered into the buffer droplet.

2.4 The AFM Settings

We used a Molecular Imaging Pico Scan III. This AFM was designed to image samples in fluid. We used a silicon nitride tip with a width of 8 nm and a force spring constant of $0.6\frac{N}{m}$. The AFM was used in tapping mode. For these samples, the cantilever was generally driven between 15 kHz and 30 kHz.

The samples were imaged in the buffers. This is advantageous for imaging biological samples, as a fluid environment mimics the environment inside a cell. After the sample is prepared the tip is lowered into the fluid droplet on the mica surface.

One must form a meniscus between the sample and the optics of the AFM so that the laser can travel unimpeded to the cantilever.

2.5 Imaging the Ribosomes

We first imaged the 50S ribosome subunit. 50S ribosome subunits were placed in buffer solution on a mica surface. The sample was then imaged with the AFM. Then the 30S subunit was imaged with the AFM. Finally the 70S ribosome, consisting of 30S and 50S bound together, was imaged. All the ribosome samples were imaged using the reconstitution buffer. The 70S ribosomes were also imaged using the tight binding buffer. The tip needs to be slowly moved across the sample to minimize moving around the ribosomes. Half a line a second is an appropriate speed.

The first attempts to image the ribosomes led to many large bumps of inconsistent height. It was was difficult to identify what was on the sample. We concluded that the concentration of ribosomes was so high that there were many layers of ribosomes piled up on each other. Vanzi et al. had also mentioned high concentrations of ribosomes on a mica surface. [13] Using a concetraion of one nM proved to be effective.

2.6 The misfortune of RNase

After creating the appropriate concentration, we ran into problems with the ribosomes breaking up into a microscopic mush. In a fiven sample ribosomes were first imaged with consistent heights, but as the scan continued the ribosomes broke up into many pieces. We discovered that if the temperature was kept around 5°C the ribosomes tended to stay intact for several hours. The problem appeared to be a contamination of RNase. RNase is an enzyme that breaks down RNA. As ribosomes contain RNA,

the presence of RNase prevented imaging ribosomes. If the buffers and the samples are kept very clean, it is possible to keep RNase contamination sufficiently low to image a sample.

We concluded it was necessary to handle the ribosomes with extreme care. We autoclaved the buffer used to store and image the ribosomes. This involves putting a prepared buffer into an oven that fills with high pressure steam. Over an extended period of time the heat denatures the RNase and any other proteins or enzymes that could harm the sample.

One must make sure that all equipment is very clean. The parts of the AFM that touch the sample must be cleaned before every use. Also, the Eppendorf tubes and micropipet tips used to prepare the sample had to be new and certified as RNase clean.

To prevent RNase contamination we revamped our storage technique. When we started imaging ribosomes we had a beaker of ribosome solution stored in the refrigerator. This is not conducive to cleanliness and any RNase in the sample will eventually destroy it. Instead, we prepare a large number of individual samples in eppendorph tubes and flash freeze them in dry ice and ethanol. The ribosomes were then stored in the -80°C freezer. When a sample was needed it could be retrieved from the -80°C, and there was no doubt as to the cleanliness or intactness of the sample. One also conserves the sample with this technique, as you only use as much sample at any given time as needed.

2.7 Binding the ribosomes to a surface

Another recurring problem consists of ribosomes not properly binding to a surface. Proteins spontaneously bind to a mica surface but RNA does not. Since ribosomes

are mostly RNA they do not have a great affinity to a mica surface. As we tapped our tip along the surface it would detect the ribosome on the first few passes and then knock it out of place, preventing us from getting a good image.

We learned to functionalize the surface with poly-L-lysine. Heéctor Becerril from the chemistry and biochemistry department at Brigham Young University suggested we use Poly-L-lysine. It creates a positive charge on the mica surface. It was initially used to bind cells onto surfaces and it is still commonly used to bind cells onto microscope slides. [20]

Poly-L-lysine was diluted in water to 1 mM concentration. We applied the poly-L-lysine to a freshly cleaved mica surface and allow it to incubate for ten minutes. Waiting longer did not increase the effectiveness of poly-L-lysine. The poly-L-lysine solution was then rinsed off of the mica surface with water and air dried. The ribosome sample was then applied to the surface.

Instead of using poly-L-lysine, the AFM probe could be moved along the sample extremely lightly and slowly. This alternative method has the advantage of not using a chemical to change the surface as this might cause some changes in the ribosome. On the other hand, it takes at least twice as long to obtain an image and is difficult to do as the ribosomes have a tendency to wander around the sample. Both methods were used to obtain images of the ribosomes.

2.8 Dissociation of Subunits

Finally, there appeared to be a problem with the ribosomal subunits staying bound together. We used the ribosome resuspension buffer to image the ribosomes. This buffer is a loose binding buffer. The ribosome subunits have no incentive to stay bound together. Dr. Jamie Williamson at Scripps encouraged use to use a tight

binding buffer. This new buffer appears to bind the subunits together more effectively, though not all the subunits appeared to be bound together in the AFM images of the 70S ribosome samples.

Chapter 3

Results and Analysis of Data

3.1 Results

I was able to consistently image the 30S, 50S, and 70S ribosome. Figure 3.1 is an image of the 50S ribosome subunit at the concentration of one nanomolarity in the ribosome resuspension buffer. When looking at an AFM image the lighter the point, the taller the point. Figure 3.1 contains consistent heights and an apropriate density of 50S ribosomes. Figure 3.2 is a chart constructed from the maximum heights of the bumps from figure 3.1. This data has been modified not to include small bumps.

Figure 3.3 and figure 3.5 displays images of the 30S ribosome subunit at one nanomolarity in ribosome resuspension buffer. Figure 3.5 contains an interesting tip artifact that causes all the ribosomes to have an interesting look. A tip artifact is caused by a tip that is broken or has something attached to it. While this makes the lateral sizes less relevent the heights are just as accurate. Figures 3.4 and 3.6 respectively are heights taken from figures 3.3 and 3.5. These charts are the full spectrum of heights found on the respective samples.

Figure 3.7 is an image of the 70S ribosome at 0.1 nanomolarity. This sample was

in the tight-binding buffer. Figure 3.8 displays the height information taken from this image. The height data represents the whole distribution of heights found in the image.

This data is not very high resolution. The AFM with the tips used in these samples has a 7 nm lateral resolution. The vertical resolution is about 3 Å.

3.2 Analysis of Data

Using the protein data bank found at www.rcsb.org, the crystal structures of the ribosome subunits were analyzed from different viewpoints estimate approximate sizes of the subunits. File 2awb from the protein data bank [21] I obtained the data on the 50S ribosome with 3.0 Å resolution. The data, for the 30S ribosome subunit with 11.1 Å resolution is file 1vox from the protein data bank. [22] Using this data the hight ranges should be from 15.4 nm to 25.4 nm for the 50S subunit and from 10.4 nm to 23.2 nm the 30S subunit.

The 50S ribosomes have consistent heights between 13.0 nm and 15.0 nm. They are easily characterized as 50S ribosome subunits. The 30S ribosomes are not as easily characterized. They have greater variation in their heights of 6.0 nm to 10.0 nm. The discrepancy from the expected values could be from the fact that we imaged ribosomes in fluid, and ribosomes are compressible. We are likely to distort the ribosomes somewhat by applying a force with the AFM tip. It appears that the most compression occurred with the 30S ribosomes. On the other hand one would expect the near 100 percent differences in heights from the asymmetry of the 30S subunit. The great uniformity of the 50S ribosomes suggests that one side has an affinity to attach to a mica surface. It appears from the heights that the 50S ribosome bind to the surface in such a way as to minimize its height.

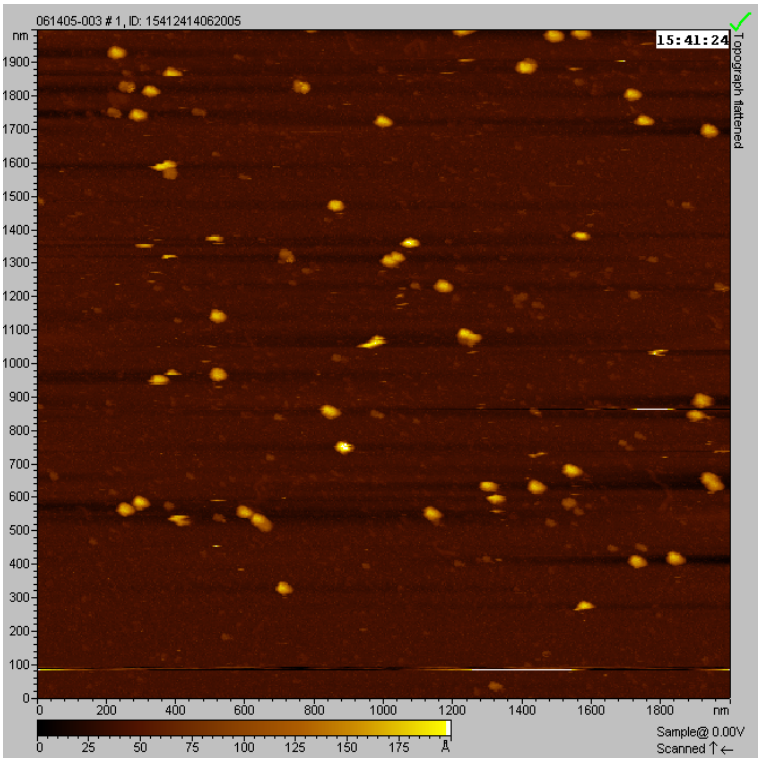

Figure 3.1 AFM flattened topography image of 50S ribosomes

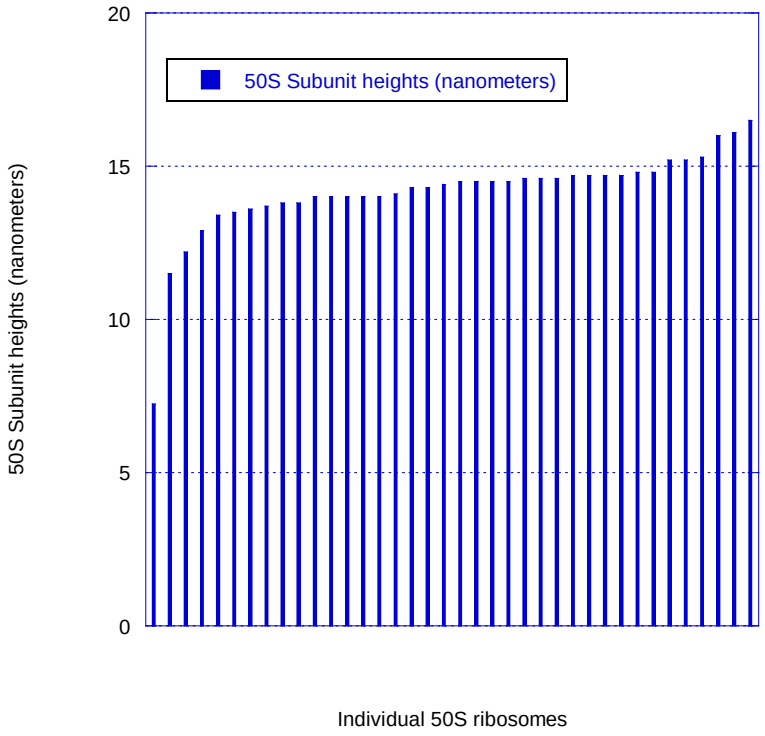

Figure 3.2 Maximum vertical higths of 50S ribosomes

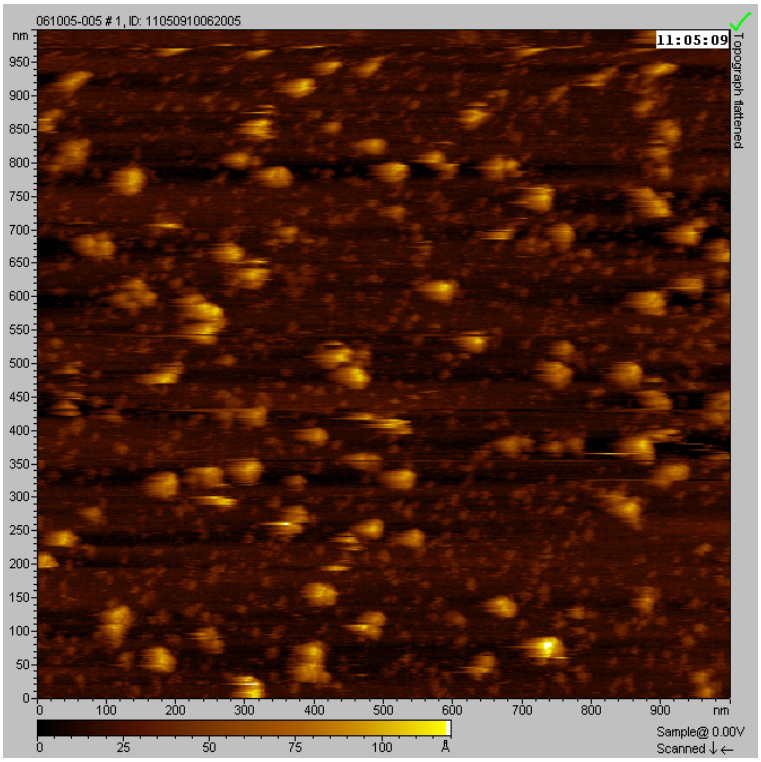

Figure 3.3 AFM flattened topography image of 30S ribosomes

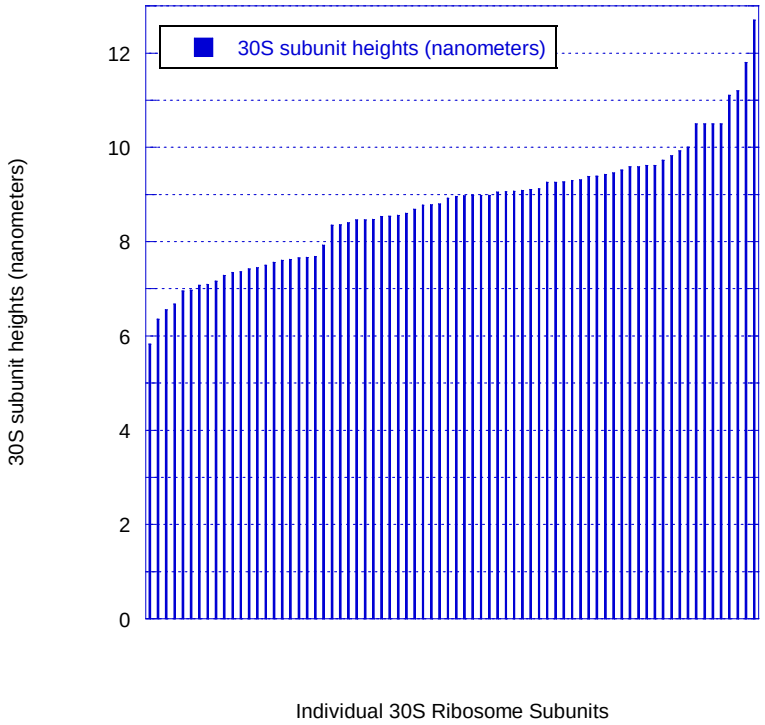

Figure 3.4 Maximum vertical heights of 30S individual ribosome subunits

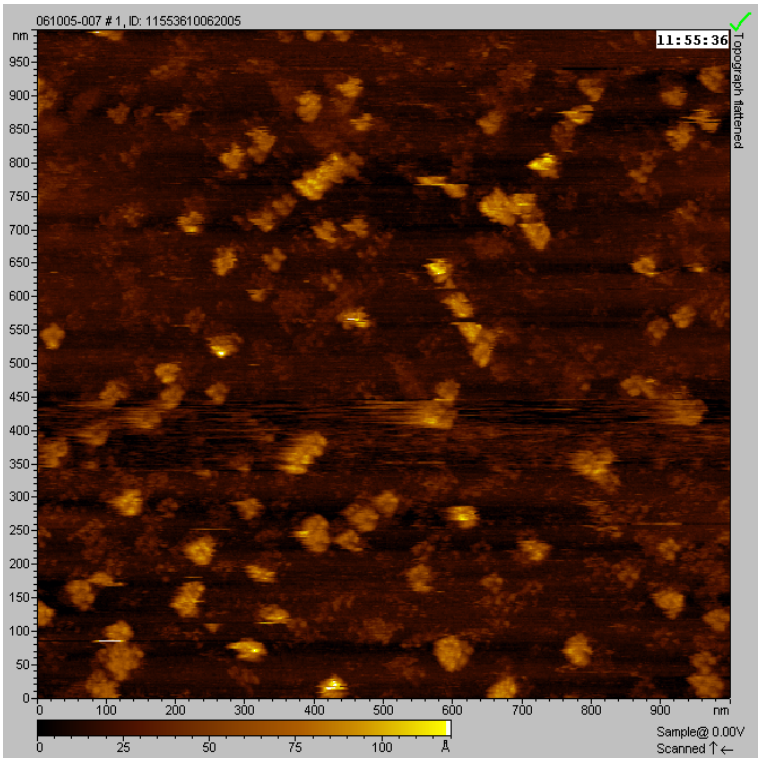

Figure 3.5 AFM flattened topography image of 30S ribosomes

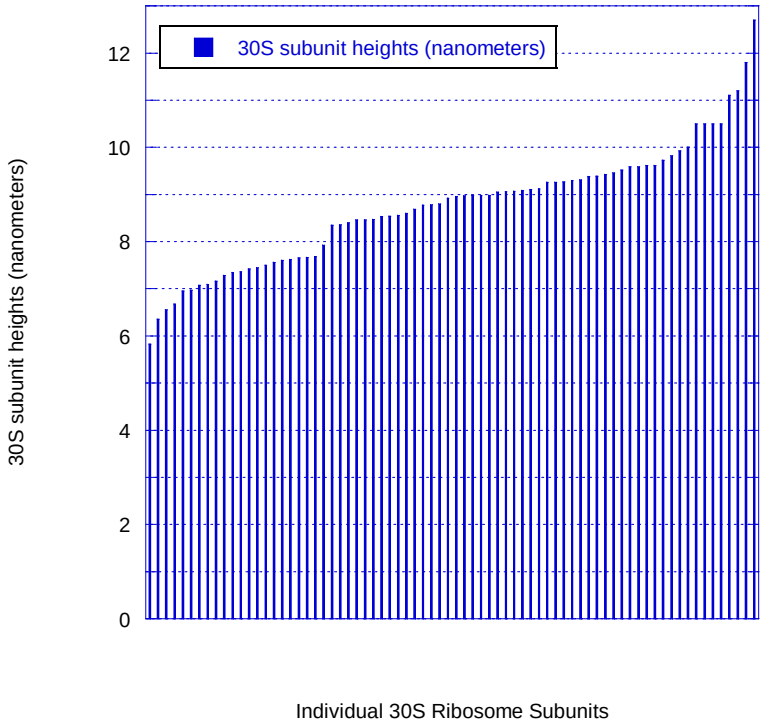

Figure 3.6 Maximum vertical heights of individual 30S ribosome subunits

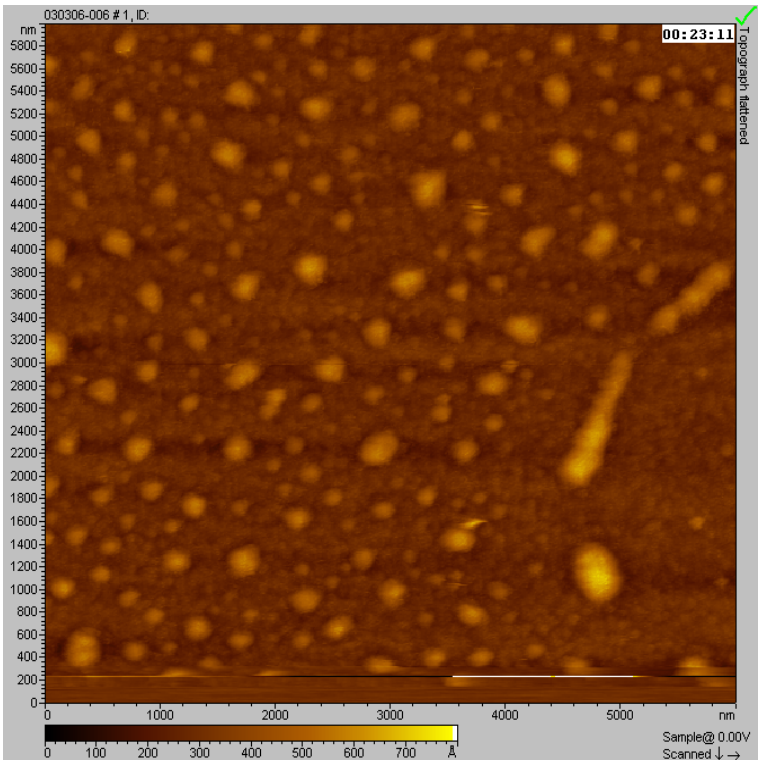

Figure 3.7 AFM flattened topography image of 70S ribosomes

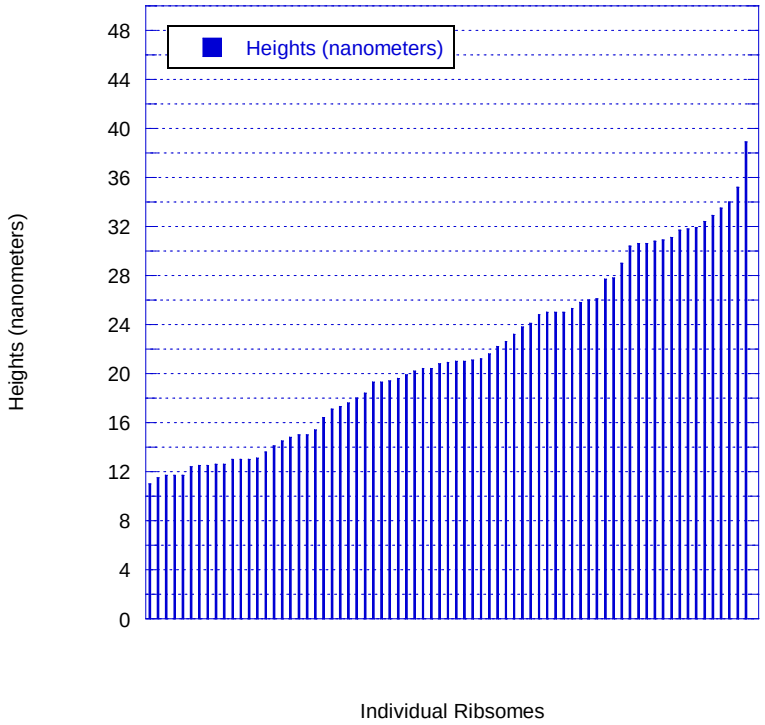

Figure 3.8 Maximum vertical heights of individual 70S ribosomes

The 70S ribosomes appears to have a wider distribution of heights. This was not fully anticipated. There is a continuum of heights from 11.0 nanometers to 35.0 nm. First it appears that not all of the ribosomes are bound together as discussed in section 2.8. This explains a fuller continuum than if it was just pure 70S ribosomes. The plateau around 12 nm is in accordance with the crystallography data for the 30S subunits, and we suspect that this represents a subset of the particles in dissociated state. The reason why the 30S subunit data matches better in this image than in the samples with only 30S subunits could be due to the different buffers. The 30S ribosomes were imaged in the ribosome resuspension buffer and the 70S ribosomes were imaged in the tight binding buffer. The range from 12 nm peaks to 25 nm peaks very likely represent 30S and 50S subunits bound to the surface in various ways. The final set of heights greater then 25 nm are probably the 70S ribosomes.

3.3 Further Studies

These imaging techniques are an essential introductory work to allow our group to further research ribosomes with the AFM. There are several fascinating areas of study that our group is pursuing that lead from this research.

Further works needs to be done imaging the ribosomes. The tip in this project was not a super tip. A super tip is a tip with a radius smaller than 1.5 nanometers, which could yield superior resolution. Also one ought to bind various ribosome inhibiting factors to the ribosomes during the imaging process to see where they bind to the ribosome.

Self-assembly is an important concept in nanotechnology. If the proper mixture of proteins and RNA are mixed together, they could form ribosomes. The AFM is well suited to study the ribosome as it self-assembles. We are currently imaging small

parts of the 30S ribosomes and trying to understand how they fold up to create a very important part of the 30S ribosome.

Ribosomes undergo conformational changes. It is possible that the AFM could be used to probe these conformational changes. By resting a tip on an individual ribosome and measuring how the tip moves one would be able to measure the conformational changes. An understanding of these conformational changes and in particular the normal modes of a ribosome would assist in bettering the current models. These models are key in understanding how ribosomes work. [16]

As ribosomes are able to build proteins, we have the goal of attaching metal groups to amino acids. Then a ribosome can be used to string together these modified amino acids in a particular order dictated by the information in the mRNA. This way we would be able to build a nano-device with specific ordering of the metal groups. In theory one could build a wire that contained an iron atom then a cobalt atom then another iron atom.

Bibliography

[1] P. B. Moore and T. A. Steitz, "The Structural Basis of Large Ribsomal Subunit Function," Annu. Rev. Biochem. **72,** 813–850 (2003).

[2] N. O. Kjeldgaard and K. Gausing, "Regulation of Biosynthesis of Ribosomes," Ribosomes **1,** 369–392 (1974).

[3] R. Loftfeild, "The Frequency of Errors in Protein Biosynthesis," Biochem. J. **89,** 82–92 (1963).

[4] C. G. Kurland, "Translational Accuracy and the Fitness of Bacteria," Annual Review of Genetics **26,** 29–50 (1992).

[5] N. Kresge, R. D. Simoni, and R. L. Hill, "George Emil Palade: How Sucrose and Electron Microscopy Led to the Birth of Cell Biology," The Journal of Biological Chemistry **280,** 19–21 (2005).

[6] B. T. Wimberly, D. E. Brodersen, W. M. C. Jr, R. J. Morgan-Warren, T. H. Andrew P. Carter, Clemens Vonrhein, and V. Ramakrishnan, "Structure of the 30S ribosomal subunit," Nature **407,** 327–339 (2000).

[7] D. N. Wilson and K. H. Nierhaus, "Ribosome Stucture and Translation, The Ribosome through the Looking Glass," Angew. Chem. Int. Ed. **42,** 3464–3486 (2003).

[8] N. Ban, P. Nissen, J. Hansen, P. B. Moore, and T. A. Steitz, "The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution," Angew. Chem. Int. Ed. **42,** 3464–3486 (2000).

[9] I. S. Gabashvili, R. K. Agrawal, C. M. T. Spahn, J. F. Robert A. Grassucci, Dmitri I. Svergun, and P. Penczek, "Solution Structure of the E. coli 70S Ribosome at 11.5 A Resolution," Cell **100,** 537–549 (2000).

[10] J. Frank and Agrawal, "A ratchet-like inter-subunit reorganization of the ribosome during translocation," Nature **406,** 318–321 (2000).

[11] M.Q.Li, "Scanning probe microscopy (STM/AFM) and applications in biology," Appl. Phys. A **68,** 255–258 (1999).

[12] M.-Q. Li, L. Xu, and A. Ikai, "Atomic force microscope imaging of ribosome and chromosome," J. Vac. Sci. Technol. B **14(2),** 1410–1412 (1996).

[13] F. Vanzi, S. Vladimirov, C. R. Knudsen, Y. E. Goldman, and B. S. Cooperman, "Protein synthesis by single ribosomes," RNA **9,** 1174–1179 (2003).

[14] X. hua Wu, W.-Y. Liu, L. Xu, and M. quan Li, "Topography of Ribosomes and Initiation Complexes from Rat Liver as Revealed by Atomic Force Microscopy," Biol. Chem. **378,** 363–372 (1997).

[15] H. Gao *et al.*, "Study of the Structural Dynamics of the E. colo 70S Ribosome Using Real-Space Refinement," Cell **113,** 789–801 (2003).

[16] J. Trylska, V. Tozzini, and J. A. McCammon, "Exploring Global Motions and Correlations in the Ribosome," Biophys J BioFAST (2005).

[17] F. Tama, M. Valle, J. Frank, and C. L. B. III, "Dynamic reorganization of the functionally active ribosome explored by normal mode analysis and cryo-electron microscopy," PNAS **100,** 9319–9323 (2003).

[18] B. Drake, C. B. Prater, A. L. Weisenhorn, S. A. C. Gould, T. R. Albrecht, C. F. Quate, D. S. Cannell, H. G. Hansma, and P. K. Hansma, "Imaging Crystals, Polymers, and Processes in Water with the Atomic Force Microscope," Science **246,** 1586–1589 (1989).

[19] H. Gorisch, D. J. Goss, and L. J. Parkhunt, "Kinetics of Ribosome Dissociation and Subunit Association Studied in a Light-Scattering Stopped-Flow Apparatus," Biochemistry **15,** 5743–5753 (1973).

[20] D. Mazia, G. Schatten, and W. Sale., "Adhesion of Cells to Surfaces Coated with Polylysine," Journal of Cell Biology **66,** 198–200 (1975).

[21] B. Schuwirth, M. Borovinskaya, C. Hau, W. Zhang, A. Vila-Sanjurjo, J. Holton, and J. Cate, "Structures of the bacterial ribosome at 3.5 Å resolution," Science **310,** 827–834 (2005).

[22] V.-S. A., B. Schuwirth, C. Hau, and J. Cate, "Structural basis for the control of translation initiation during stress.," Nat.Struct.Mol.Biol **11,** 1054–1059 (2004).

Index