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Atomic Force Microscopy: An Enabling Nanotechnology for Diabetes Research

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List of Abbreviations

AGEs – Advanced Glycation End-Products

AFM – Atomic Force Microscopy

FM – Frequency Modulation

PBS – phosphate buffer saline

SPM – Scanning Probe Microscopy

STM – Scanning Tunneling Microscopy

Abstract

Atomic force microscopy (AFM), a scanning probe microscopy-based technique with atomic resolution and piconewton force sensitivity, has been an enabling tool for nanotechnology and nanobiotechnology since its invention in 1986. In AFM, a nanometer-sized probe tip attached to the end of a microfabricated cantilever is scanned near or in contact with a surface in order to construct an image and to determine various properties of the surface by measuring the interaction force between the tip and the surface. AFM can be operated in air and liquid and can be applied to soft biological samples in physiological environments. Thus, it has vast applications in the developing field of nanomedicine. Diabetes is a group of diseases characterized by abnormally high blood glucose resulting from insulin resistance and/or impaired or absent insulin secretion, and the field of nanomedicine can contribute in terms of understanding the development and progression of the disease and also in designing nanotherapies to treat the disease. In this chapter, we discuss the role of AFM in understanding and designing therapies for diabetes and the impact of AFM on diabetes research, from the nano- to the microscale, and from single molecules to macromolecular assemblies and individual cells. In particular, the role of AFM in understanding pathophysiological changes and the pathology of the disease, and the role of AFM in the treatment of diabetes are presented.

1. Introduction

Recent advances in nanotechnology have been enabled by tools capable of fabricating and imaging nanoscale features, measuring materials properties at the nanoscale, and manipulating nanosized objects. One family of techniques in particular, namely, scanning probe microscopy (SPM), has made a significant impact on a wide variety of fields due primarily to its

versatility, resolution, and sensitivity. The field of SPM has evolved from the inventions of the scanning tunneling microscope (STM) in 1982 (Binnig et al. 1982) and the atomic force microscope (AFM) in 1986 (Binnig et al. 1986) to an impressive measurement and manipulation platform capable of measuring chemical recognition events and, e.g., structural, mechanical, electrical, and magnetic, properties of materials, and of manipulating at the single-molecule and atomic levels. Whereas the STM can be used to construct atomic scale images of conducting materials based on the tunneling current between tip and sample, the AFM can be applied to any material, and images are constructed from the interaction force between the tip and sample. These tools have been identified as ushering in the nanometer age (Rohrer 1994) and have been essential to the development of nanotechnology, i.e., the measurement, manipulation, and exploitation of materials and materials properties at the nanoscale. Nanotechnologies applied to biology and medicine are thus nanobiotechnology and nanomedicine, and can be described as the characterization and manipulation of biological systems and the diagnosis and therapy of disease at the nanoscale using nanomaterials and nanotools. The use of nanomedicine for, e.g., biosensing, drug-design, and the design and characterization of nanomaterials for drug delivery and power-generators holds the promise for future breakthroughs in therapies and diagnostics.

Atomic force microscopy is well-suited for studying biology at the nanoscale as it has the ability to (i) image nanostructures, proteins, biological macromolecules, and cells in (ii) air, liquid, and physiological environments, (iii) measure local interaction properties and materials properties at specified positions, i.e., AFM force spectroscopy, and (iv) be integrated with complementary imaging modalities. The basic operating principles of AFM are described in

Table 1 and illustrated in Figure 1. AFM, and to a lesser extent, STM, have been applied to biological systems for some time (Hansma et al. 1988, Drake et al. 1989), but the breakthrough for imaging soft biomaterials came with the invention of dynamic AFM modes (Zhong et al. 1993) and the first application of these modes in a liquid environment (Hansma et al. 1994) and on cells in solution (Putman et al. 1994). For over two decades now, AFM has been used to study biology, as demonstrated by early review papers on spectroscopy and imaging (Rademacher et al. 1992, Hansma and Hoh 1994, Heinz and Hoh 1999) through to more recent papers specifically addressing the position of AFM in nanobiotechnology (Müller and Dufrêne 2008). Advances in AFM for biology continue to be made, particularly in the areas of frequency modulation AFM (FM-AFM) in liquid (Jarvis et al. 2008), multifrequency AFM (Proksch 2011), high-speed AFM (Ando et al. 2001, Kodera et al. 2010), and nanomedicine (Riehemann et al. 2009, Lal and Arnsdorf 2010, Sitterberg et al. 2010). One disease of particular importance for nanomedicine, with applications in diagnosis and therapy, and in understanding the mechanisms of the disease, is diabetes (Pickup et al. 2008).

Diabetes is a disease affecting millions of individuals throughout the world and enormous socioeconomic burdens are associated with diabetes and the treatment of its complications. Most patients with diabetes have non-insulin dependent diabetes mellitus or type 2 diabetes. Insulin dependent diabetes mellitus is known as type 1 or juvenile onset diabetes. Both diseases are characterized by high blood glucose either because the body does not produce (enough) insulin, the hormone responsible for glycemic control, or because the body is unable to use the insulin that is produced by the body effectively. In general, normal blood glucose is maintained through the balance of insulin production and action. In type 2

diabetes, insulin insensitivity or resistance, triggered partly by environmental (e.g., obesity, overeating, physical inactivity) and genetic (i.e., heredity) factors, and pancreatic β-cell dysfunction, are key mechanisms in the development of the disease. Insulin resistance leads to increased β-cell dysfunction over time and without diagnosis and intervention, the disease will progress; early stages of insulin resistance or pre-diabetes often go undiagnosed (Harris et al. 1992). Impaired insulin secretion, loss of β-cell mass, increased β-cell apoptosis, and the presence of islet cell amyloid are also associated with type 2 diabetes (Haataja et al. 2008). Type 1 diabetes generally results from an autoimmune attack on β-cells thought to be triggered by an environmental factor in susceptible individuals, leading to β-cell destruction and complete insulin deficiency. Thus, early screening, improved insulin administration and or action, blood glucose monitoring, and effective therapies for glycemic control are important factors for treating and preventing the progression and complications of diabetes. Poor glycemic control can lead to or is associated with a variety of serious short- and long-term complications, often exacerbated by risk factors such as hypertension and high cholesterol, including retinopathy, neuropathy, nephropathy, and cardiovascular disease, which can lead to blindness, amputation, renal failure, and death. Type 1 diabetes is further associated with other autoimmune conditions such as thyroid disease. Currently, there is no cure for diabetes.

In this chapter, the applications of AFM to areas of health and disease in diabetes research are presented. In particular, the role of AFM in understanding pathophysiological changes in diabetic tissues, proteins, and cells through measurements of structural and mechanical properties are discussed in Section II; The role of AFM in understanding the pathology of the disease through the characterization of fibrils, proteins, and cells relevant to

diabetes is discussed in Section III; In Section IV, the role AFM plays in the treatment of diabetes is presented, including in the design and characterization of nanomaterials such as nanoparticles and nanocoatings, and nanodevices; Finally, in Section V, future prospects for AFM as a nanotechnology for diabetes research are outlined.

Applications to areas of health and disease

Diabetes is a disease associated with elevated blood glucose levels, which, over time can lead to a number of complications and to increased health risks. Atomic force microscopy is a nanotechnology tool with a demonstrated ability to aid in understanding pathophysiological changes and the pathology of diabetes, and to contribute to the development and characterization of nanomaterials for the treatment of the disease.

2. Role of AFM in the assessment of the pathophysiology of diabetes

Microvascular disease and neuropathy often work in tandem to prolong wound healing in patients with diabetes, which in extreme cases can lead to, e.g., foot ulcers, and result in amputation. Poor glycemic control resulting in hyperglycemia leads to normal enzyme-mediated glycosylation of the blood hemoglobin and to (non-enzymatic) glycation of the hemoglobin and of other proteins, the former of which leads to elevated glycated hemoglobin levels, while the latter can lead to the formation of advanced glycation end-products (AGEs) and impaired biomolecular function (Brownlee et al. 1984). Delayed wound healing in diabetics is often associated with the presence of AGEs, which have been further shown to be associated with changes in the mechanical properties of tissues. Furthermore, diabetes can be said to accelerate the aging process, and many changes in tissue properties attributed to aging can also manifest in patients with diabetes, including reduced elasticity in collagenous tissues. As collagen is

present throughout the body, from, e.g., skin to bones, teeth, eyes, and arterial walls, this change in the mechanical properties of collagen can have a significant impact on health. While a number of tools have been used to study structural and ultrastructural changes in tissues and cells, AFM has several key advantages in this regard as (i) AFM does not require any special sample preparation, i.e., no metal coating of the sample is required, as is the case for electron microscopy; (ii) AFM can be operated in liquid and physiological environments; (iii) AFM can be used to measure mechanical properties, such as elasticity; and (iv) AFM can be used to measure properties locally, on the nanoscale, an important consideration when dealing with complex, often hierarchically-structured biomaterials. In this section, we address the use of AFM to study the structural and mechanical properties of tissues, fibrils, membrane proteins, and cells affected by diabetes.

2.1 Tissues and fibrils

Collagen, specifically collagen from diabetic rats has been studied extensively by AFM, typically revealing structural alterations in collagen fibrils. In a study by Odetti et al., tendons from diabetic and non-diabetic rats and tendons from non-diabetic rats which had been subsequently incubated in a phosphate buffer saline (PBS) solution containing glucose for 15 days were investigated by AFM (Odetti et al. 2000). Diabetic and glucose-incubated collagen fibrils were found to have similar radii and gap depths, and larger radii and gap depths than non-diabetic collagen fibrils, as illustrated in Figure 2. The diabetic and glucose-incubated collagens were further analyzed to determine fructosomine and pentosidine levels by a commercial assay and using high pressure liquid chromatography, respectively, allowing the authors to conclude that extended exposure to glucose both *in vivo* and *in vitro* leads to

structural changes in collagen fibrils. This work provides evidence that glycation through cross-links plays a significant role in structural changes. It also further corroborates earlier work by some of the same authors addressing the role of AGEs on structural changes in collagen fibrils from aged rat-tail tendon (Odetti et al. 1998). A 2003 article by Wang et al., comparing collagen fibril structure in nerve and tendon collagens from diabetic and non-diabetic rats, revealed that collagen diameters were consistently larger for the collagens harvested from diabetic rats, while the typical 67 nm periodicity of the non-diabetic fibrils remained unchanged in the diabetic fibrils (Wang et al. 2003).

Collagen is not the only protein that may be affected by glycation, nor is it the only structural protein with an important role in microvascular disease, a secondary effect of diabetes. In particular, fibrillin, which is known to play an important role in the elasticity of healthy tissues, has also been shown to become modified in type 1 diabetic tissues (Akhtar et al. 2010). Akhtar et al. used AFM to measure structural changes in normal and diabetic fibrillin microfibrils isolated from a rat aorta, and reported that while the length of the microfibrils remained unaltered, the periodicity of the fibrils was reduced significantly in diabetic rats, an ultrastructural change thought to be related to observed changes in macroscopic mechanical properties in diabetic rat aortas.

2.2 Membrane proteins

While high-resolution AFM is capable of obtaining stunning images of, e.g., membrane proteins and lipid bilayers, it has found few applications in diabetes research. In 2009, Mangenot et al. used high-resolution AFM to address the formation of junctional microdomains from normal bovine and human cataract lens membranes (Mangenot et al. 2009). The cataract

lens was extracted from the surgery debris of a type 2 diabetes patient, while the normal lens membranes were harvested from calves. Junctional microdomains form junctions between cells in the lens facilitating the flow of ions, waste, etc. between them, allowing cells deep within the lens to be nourished, evacuate waste, and remain healthy (Donaldson et al. 2001), thus preventing the lens opacification associated with cataracts. In the normal lens membranes, it was found that aquaporin-0 formed well-registered, square-lattice array structures often bordered by connexons, as shown in Figure 3a. In the cataract lens membranes, however, the aquaporin-0 microdomains appear less continuous, forming in rows, and there is a notable absence of connexons, as can be seen in Figure 3b. The authors conclude that the lack of connexons in the cataract lens from the type 2 diabetes patient contributed to both the malformation of the aquaporin-0 microdomains and to the ability of the junctions to effectively circulate nutrients and waste, and note a similar observation in aged cataract lens membranes (Buzhynskyy et al. 2007).

2.3 Cells

A cellular-level effect of the toll of diabetes on the body has also been highlighted by AFM measurements of mechanical properties of red blood cells. Just as variations in elasticity in red blood cells infected with the *Plasmodium falciparum* parasite responsible for malaria have been observed by micropipette aspiration (Suresh et al. 2005), highlighting a possible route for nanodiagnostics of disease, several groups have reported increased stiffness of diabetic red blood cells (Fornal et al. 2006, Hekele et al. 2008, Jin et al. 2010). The observation that erythrocytes from type 2 diabetic patients have greater Young's moduli, and thus lower elasticity, is particularly troubling when taken with the knowledge that the same patients likely

suffer from arterial stiffening as a result of ultrastructural changes in collagen and/or fibrillin; the combination of the two further impairs circulation, thus increasing wound healing time.

3. Role of AFM in understanding the pathogenesis and pathology of diabetes

As discussed previously, AFM has been used extensively to address problems of biological interest, and in limited cases, to directly assess the structural and mechanical changes associated with diabetes. Type I and 2 diabetes are both associated with β-cell dysfunction or destruction, although the exact mechanisms remain unknown. The long list of secondary diseases and complications associated with diabetes suggests the importance of understanding the effect of high blood glucose in cells and tissues throughout the body, in particular in the pancreas and kidneys. As AFM has been applied in a wide variety of biologically-relevant studies, including cell mechanotransduction, lipid bilayer and raft formation, protein membrane and channel formation, protein misfolding and fibrillization, binding forces between molecules, and protein nanomechanics, AFM has the potential to play a significant role in understanding the development and effect of diseases such as diabetes. In this section, we discuss the use of AFM to study tissues relevant to diabetes.

3.1 Fibrils

Both insulin and amylin, or islet amyloid polypeptide, are peptide hormones secreted by islet β-cells in the pancreas, and both peptides are known to fibrillate under certain conditions to form structurally similar amyloid fibrils. The formation of amyloid fibrils is associated with many neurodegenerative diseases and the fundamentals of protein folding and misfolding have been the topics of extensive research (Dobson 2003, Chiti and Dobson 2006). In relation to diabetes, amyloid deposits have been found in pancreatic islet β-cells of type 2 diabetes

patients (and elsewhere including the cataract lens exterior) and are considered a pathological hallmark of the disease, perhaps contributing to the dysfunction of the β-cells or merely a byproduct of the disease (Haataja et al. 2008). Additionally, amylin amyloid fibrils have been shown to be toxic to cultured β-cells (Lorenzo et al. 1994). Understanding the conditions leading to the formation of such fibrils and unraveling the structure of such deposits are important steps in determining the role amyloid fibrils play in the progression of diabetes, a course of study aptly suited for AFM.

Goldsbury et al. studied the assembly of individual amylin fibrils formed from synthetic human amylin *in vitro* on an atomically-flat substrate using time-lapse AFM (i.e., by recording sequential images) (Goldsbury et al. 1999). Unlike electron microscopy techniques which require dehydrated fibrils, AFM measurements in liquid allow the fibrils to be visualized in physiologically relevant conditions, and furthermore, time-lapse AFM allows the dynamics of the formation process (i.e., the growth rate) to be measured. The authors report that both fibrils formed in a test tube and subsequently deposited onto a substrate and those assembled directly onto the substrate are polymorphic, likely consisting of multiple protofibrils. Time-lapse AFM studies further reveal that while the fibrils grow bidirectionally on a surface and with similar growth rates, as shown in Figure 4, the fibrils are less coiled than those prepared in a test tube and the density of protofibrils is higher, presumably due to the interaction with the surface. Furthermore, controlled tweaking of the growth environment could reveal which conditions accelerate or retard formation, leading to targeted therapies. Sedman et al. also looked at the formation of amylin amyloid fibrils formed *in vitro* for 6 and 18 hours, looking specifically at a stretch of residues in amylin (20-29) thought to be crucial to fibril formation

(Sedman et al. 2005). The kinetics of human insulin amyloid fibril formation has also been addressed by AFM (Jansen et al. 2005) and using time-lapse AFM (Podestà et al. 2006) and also as a function of temperature and insulin concentration at low pH (Mauro et al. 2007). More recently, Fukuma et al. used liquid FM-AFM to determine the surface structure of isolated amylin amyloid fibrils with Ångström resolution (Fukuma et al. 2008). As shown in Figure 5, the results reveal the cross-β strand structure of amylin fibrils.

Glucagon is also an amyloid fibril forming hormone secreted by the pancreas that is involved in the regulation of blood glucose. The fibrillation process has been studied by AFM (Dong et al. 2006, De Jong et al. 2006), and force spectroscopy measurements have revealed information about the amyloid structure (Dong et al. 2008) and glucagon/anti-glucagon binding (Lin et al 2007). Glucagon preparations are prescribed as a treatment of hypoglycemia. In general, amyloid fibril formation and functional properties of amyloid have been intensely studied by AFM adding to the understanding of the role of amyloid in diabetes.

3.2 Protein-membrane interactions

While amyloid deposits are typically found in islet cells of patients with type 2 diabetes, and amyloids have been identified with toxicity, it is not yet clear to which aggregation state the toxicity can be attributed (Dobson 2003). Green et al. used time-lapse AFM to demonstrate that the amylin peptide penetrates and disrupts mica-supported lipid bilayers, as shown in Figure 6, and thus membrane-permeant oligomers may be responsible for cytotoxicity (Green et al. 2004). Cho et al. reported the formation of amyloid deposits after exposing a lipid bilayer to amylin (Cho et al. 2008).

3.3 Cells

While diabetes is associated mainly with β-cell dysfunction, very few attempts at β-cell imaging by AFM have been made (Girasole et al. 2007), however, high blood glucose can affect the functioning of other cells and the means by which cells interact with their environment. It has recently been shown that pancreatic acinar cells can be reprogrammed *in vivo* into insulin producing β-cells (Zhou et al. 2008, German 2008). Living acinar cells have also been imaged by AFM, enabling the membrane dynamics of exocytosis to be visualized (Schneider et al. 1997, Cho et al. 2002). Exocytosis is the process by which a cell moves the contents of secretory vesicles across the cell membrane, including insulin and amylin in β-cells, and furthermore, the expression of exocytotic proteins can be impaired by diabetes (Abdel-Halim et al. 1996). To investigate impaired membrane fusion in the liver of rats with chronic pancreatitis, a condition which is related to or may lead to diabetes, the binding forces of hepatic microsomal and plasma membrane proteins in normal and pancreatitic rats has also been studied by AFM force spectroscopy (Slezak et al. 1999). The kidney is responsible for filtering waste from blood, and kidney disease is common in patients with diabetes. Recently, decreased insulin action in kidney podocytes, a type of epithelial cell, has been linked to diabetic complications (Welsh et al. 2010, Rask-Madsen and King 2010). Podocytes have been imaged in tissue sections by AFM (Ushiki et al. 1994), and the elasticity of epithelial cells has been shown to decrease with aging (Berdyyva et al. 2005).

4. Nanotherapies: Role of AFM in the treatment of diabetes

Diabetes treatments generally involve lifestyle and drug therapies. For type 2 patients, the drugs are designed to improve the effectiveness of the insulin secreted by the islet cells, and in some cases, additional insulin is prescribed, while in type 1 patients, the primary therapy is

the administration of insulin. A key component to delivering correct dosages is implementing an effective closed feedback loop of glucose measurement and insulin action (Imanishi and Ito 1995, Hovorka 2005), in effect replacing the role of the dysfunctional and or destroyed islet cells. In this section, the role of AFM in the development of therapies related to glucose monitoring and sensing, and to insulin delivery are presented. Clearly, AFM has been employed to study the size, structure, and properties of many nanomaterials related to diabetes research discussed in this book, but this discussion is largely beyond the scope of this chapter.

4.1 Glucose Sensing

Ideally glucose monitoring should be continuous and non-invasive (Pickup et al. 2005). The development of glucose biosensors is an active field (Wang 2008) and AFM has or can play a role in many aspects, from understanding binding forces involved in glucose biosensing to characterizing the nanomaterials and coatings used in glucose biosensing. Notably, several attempts have been made to fabricate glucose sensors directly onto AFM tips (de Souze Pereira 2000, Kueng et al. 2005). In addition, glucose/galactose receptor binding has been studied by AFM force spectroscopy revealing a change in stiffness in the activated form, which may be relevant for glucose biosensor design and implementation (Sokolov et al. 2006).

Layer-by-layer assembly (Johnston et al. 2006), the assembly of oppositely charged polyelectrolyte or polymer layers, can be investigated using AFM, which has been employed extensively for the study of polymers and is sensitive to electrostatic forces. Glucose sensors have been prepared via a layer-by-layer approach (Ferreira et al. 2004, Zhao et al. 2005). Other approaches for the development of implantable glucose biosensors incorporate nitric-oxide xerogel microarrays into polymer membranes and layers (Oh et al. 2005). Nitric oxide is an

effective inhibitor of platelet and bacterial adhesion, and such devices may exhibit improved biocompatibility and lifetimes (Oh et al. 2005).

4.2 Insulin

Insulin is generally delivered, primarily in type 1 diabetic patients, as a solution or a suspension subcutaneously via syringe or an extracorporeal insulin pump, or through an insulin pump implanted in the peritoneal cavity. Transdermal, intranasal, pulmonary, and oral routes are at various stages of development or are commercially available. One limitation of insulin delivery is that it does not effectively mimic the patterns of insulin secretion in the body, making it difficult to achieve good glycemic control. As discussed in this subsection, significant efforts have been made in designing insulin for tailored properties and delivery routes, while also designing encapsulation approaches for coating insulin for delivery and islet cells for transplantation.

AFM has been used to study the self-association and crystallization of insulin formulations which have been modified by specific alterations of wild-type insulin to reduce monomeric association and influence self-assembly to develop, e.g., extended time action microcrystalline insulin suspension therapies. The rate of dissociation to the bioactive insulin monomer will directly affect the pharmacokinetics of the insulin and will depend on the structure of the crystals, which can be determined using AFM (Yip et al. 1998a). Yip et al. have released a series of papers using time-lapse AFM to study the influence of sequence variation and insulin analogs on insulin crystallization, as shown in Figure 7 (Yip et al. 1996, 1998a, 1998b, 2000). Time-lapse AFM has also been used to study the morphology and growth of bovine insulin crystals (Waizumi et al. 2003). Interestingly, Yip et al. have additionally studied the

mechanisms of insulin dissociation using AFM force spectroscopy to probe insulin monomer-monomer binding, which is crucial to understanding the efficacy of insulin therapies, observing both dimer dissociation and disentanglement events in the force curves (Yip et al. 1998c).

Many formulations of insulin, including encapsulated or coated nanoparticles, have been considered for alternative, i.e., oral, delivery routes (Fan et al. 2006, Ye et al. 2006). AFM can be used to image nano- and nanoencapsulated particles, providing information on the process of nanoparticle formation and encapsulation processes (Olivia et al. 2002, 2003), nanoparticle size, nanoparticle degradation, etc. Sitterberg et al. have written a recent review of the uses of AFM for characterizing nanoscale drug delivery systems (Sitterberg et al.).

While these efforts address the delivery of insulin, the development of a bioartificial pancreas (Kizilel et al 2005, Opara et al. 2010) via, e.g., islet nanoencapsulation, holds promise for long-term treatment of type 1 diabetes (Beck et al. 2007). To address the problem of rejection of transplanted islet cells, nanoencapsulation has developed as a method to achieve immunoisolation. The material should be biocompatible, and isolate the cells from the immune system while still permitting normal exocytosis and endocytosis. AFM has been used to characterize such micro- to nanoencapsulation devices (Xu et al. 1998) and multilayer nanoencapsulation has been used to coat islet cells (Krol et al. 2006).

5. Future Prospects

While AFM has already made a significant impact on diabetes research, there are several areas where further developments are expected, particularly in live cell imaging, in imaging dynamic processes with high-speed AFM, in correlating structure and function, and in nanocharacterization support of the vast efforts in the design of biosensors, drugs, drug delivery

systems, nanoencapsulation and other nanodiagnostic and nanotherapy approaches. Combined AFM and confocal imaging approaches may help understand signaling processes associated with diabetes, and allow mechano- and electrotransduction in relevant cells to be studied. AFM recognition imaging for visualizing binding sites and high-speed AFM for imaging dynamic processes, such as fusion pore formation, have potential applications as well. Ultra high-resolution AFM has potential applications in understanding interactions between sugars and cell membranes and for visualizing the interfacial structure at the surface of insulin microcrystals. Additionally, by combining complementary AFM techniques for measuring and comparing elasticity, viscoelasticity, electromechanical coupling, and electrostatic interactions of diabetic and healthy tissues, differences between the tissues can be better understood. Notably, electromechanical coupling has already been studied at the nanoscale in amyloid fibrils in a liquid environment (Kalinin et al. 2007, Nikiforov et al. 2010).

6. Summary

Atomic force microscopy has found many applications in nanotechnology and nanomedicine including the understanding of diabetes, a disease with increasing incidence rates affecting millions of people. In particular, AFM has been used to observe ultrastructural differences in collagenous tissues and differences in mechanical properties of red blood cells between diabetic and non-diabetic animals and patients. These observations provide insight into the effect diabetes has on the entire body and stress the importance of glycemic control in preventing long-term complications. While the results suggest AFM could be used for diagnosis and monitoring the progression of the disease, there are more suitable techniques. AFM has also been used to investigate the role the amylin peptide and amylin fibrils has on diabetes and

has the potential for revealing the relationship between amylin and β-cell dysfunction. AFM has also played a key role in the development of nanoparticles and nanoencapsulation for biosensing and drug delivery, and in understanding and designing different insulin formulations for less-invasive insulin delivery and rapid or slow acting insulin. In the 25 years since the birth of AFM there have been many contributions to the fields of nanotechnology and nanomedicine, and with the constant evolution of novel imaging modes and improved instrument and probe design, AFM will continue to make a significant impact.

Acknowledgement

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Summary points

- AFM can be operated in physiological environments and can be combined with optical microscopy techniques making it applicable to a wide variety of topics in biology and nanomedicine.
- Prolonged exposure to high glucose levels as a result of diabetes can lead to ultrastructural changes in collagen fibrils that have been visualized using AFM.
- AFM has revealed an increase in the stiffness of red blood cells of diabetic vs. non-diabetic patients.
- Amylin, insulin, and glucagon are all peptides which fibrillate to form amyloid fibrils. AFM has been used to visualize the formation and ultrastructure of such macromolecules, and has been used to observe the interactions of such peptide monomers with lipid bilayers.

- AFM has provided significant nanocharacterization support for the design of biosensors, drugs, drug delivery systems, nanoencapsulation and other nanodiagnostic and nanotherapeutic approaches for treating diabetes.
- AFM as a nanotechnology continues to be developed and has the potential to be an indispensable tool for understanding and treating diabetes.

Definitions

Contact mode – In contact mode AFM, the cantilever is scanned with the tip in contact with the sample surface. A feedback loop is employed which keeps a constant force between the tip and sample by adjusting the tip-sample separation allowing an image of the surface topography to be recorded.

Force Spectroscopy – AFM force spectroscopy refers to the local measurement of the interaction force between an AFM tip and a sample as a function of the distance between the two. In the case when a molecule is attached between the tip and a substrate, the force required to break molecular bonds can be measured. When the tip is used to plastically deform a material, local elasticity can be measured. Spectroscopy can also refer to the local bias-dependent response (e.g., current, electromechanical coupling) of a sample in contact with a tip.

Glycation – Glycation is the non-enzymatic binding of a sugar molecule to a protein or lipid.

Multifrequency – Whereas in tapping mode, the cantilever is oscillated at a single resonant frequency, multifrequency AFM techniques excite the cantilever at several frequencies simultaneously or across a band of frequencies.

Tapping mode – Tapping mode or intermittent contact AFM is a dynamic AFM mode of operation whereby the cantilever is oscillated near its resonance frequency, and the interaction forces between the tip and sample are monitored using a feedback loop to keep the amplitude of the cantilever oscillation constant as it moves laterally relative to the sample surface. Tapping mode AFM reduces the lateral friction forces inherent in contact mode AFM and also overcomes the capillary forces of surfaces in air.

Table

1. AFM is a nanotechnology tool based on monitoring the interaction force between a surface and a nanometer-sized probe tip at the end of a microfabricated cantilever beam.

2. The interaction force is measured by monitoring the deflection of the AFM cantilever.

3. The deflection of the cantilever is detected using an optical beam deflection method in which a laser or laser diode light source is reflected off the backside of the cantilever and into a photodetector.

4. The photodetector thus relates the position of the reflected light to the distance the cantilever is deflected.

5. The tip and sample are brought together using a motor positioning system with course position control and a piezoelectric element with precise position control.

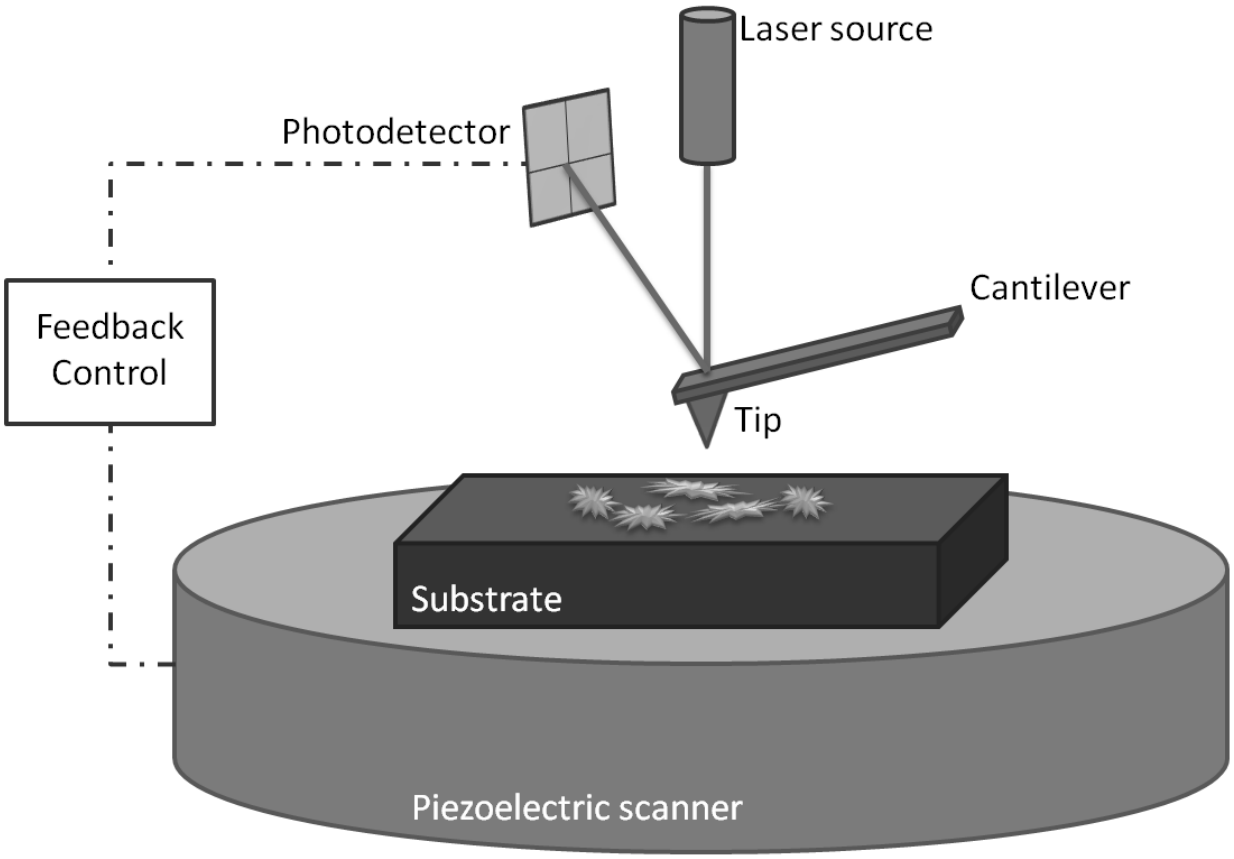
6. A feedback loop is employed which keeps, e.g., a constant force between the tip and sample by adjusting the tip-sample separation using a piezoelectric element.

7. The tip moves in a raster-scan pattern with respect to the sample allowing an image of the surface topography to be recorded.

Title of Table 1: Key Features of AFM

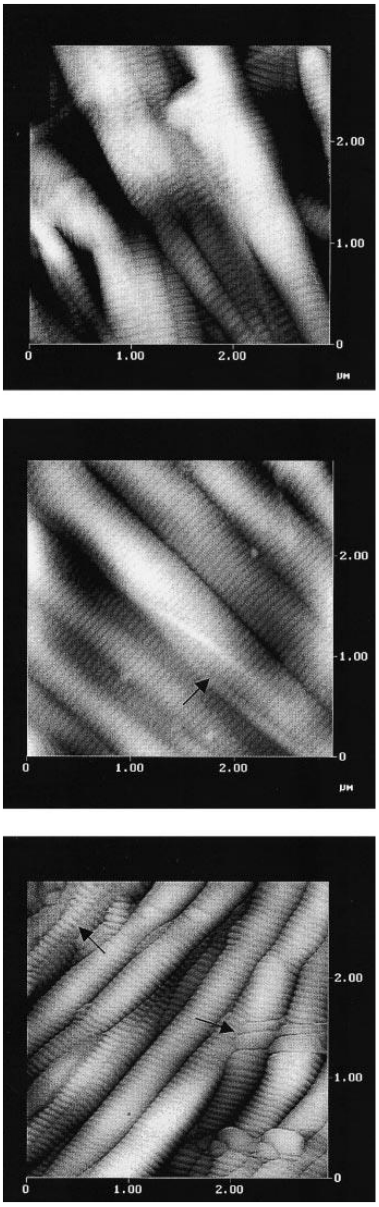
Legend to Table 1: This table lists the basic operation principles of AFM.

Figures



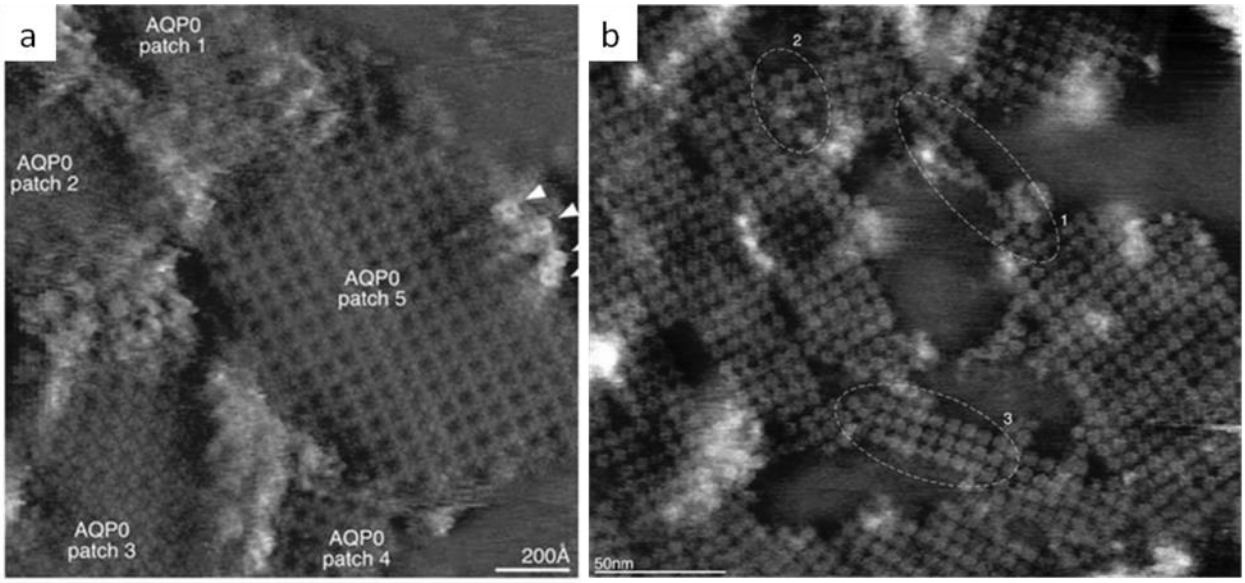
Title of Figure 1: Schematic depicting the basic components of an AFM.

Legend to Figure 1: In AFM, the cantilever deflection is measured using a photodetector to quantify changes in the position of a laser reflected off the backside of the cantilever. A feedback loop is used to maintain a constant force between the tip and the sample by adjusting the tip-sample separation using a piezoelectric scanner, which can also be used to move the sample with respect to the tip, thus allowing an image of tip-sample interaction force to be recorded.



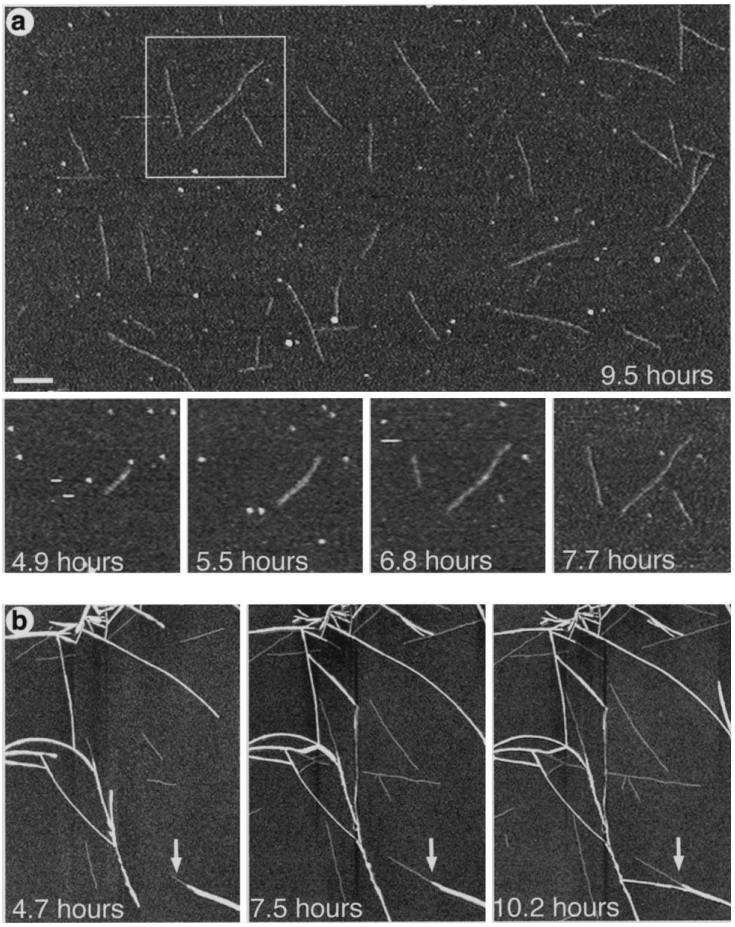
Title of Figure 2: AFM of non-diabetic, diabetic, and glucose incubated collagen.

Legend to Figure 2: AFM topography images of (a) non-diabetic, (b) diabetic, and (c) glucose-incubated rat tail tendon. The fibrils in (b) and (c) have larger diameters than those in (a). Arrows indicate the merging of fibrils. Reprinted from Odetti et al., copyright 2000, with permission from John Wiley & Sons, Ltd. (Odetti et al. 2000).



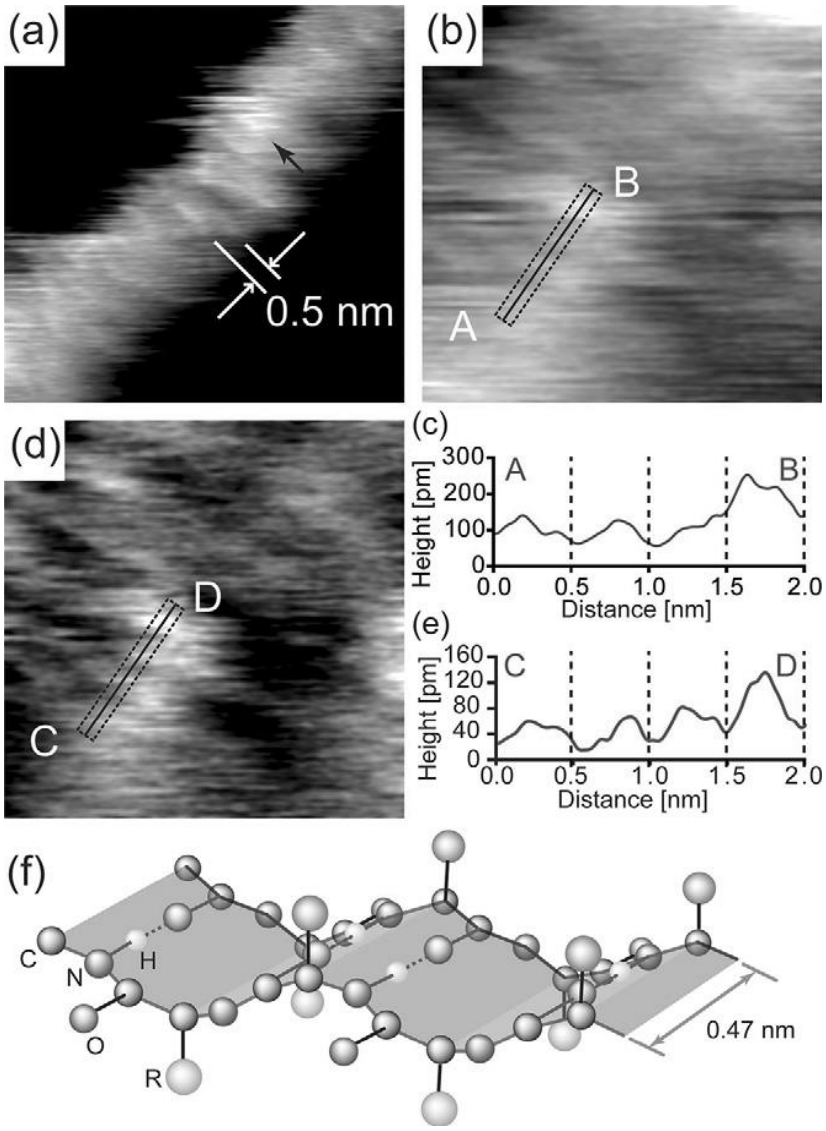
Title of Figure 3. AFM images of junctional microdomains in calf and human cataract lenses.

Legend to Figure 3: (a) AFM topography image of healthy calf lens membranes. Arrows indicate the location of connexons and aquaporin-0 patches are labeled AQP0 patch 1-5. (b) AFM topography image of membranes from a cataract lens of a patient with type 2 diabetes. Single, double, and triple rows of aquaporin-0 tetramers are highlighted with dashed-ellipses 1-3. Reproduced with permission and copyright 2008, Springer (Mangenot et al. 2009).



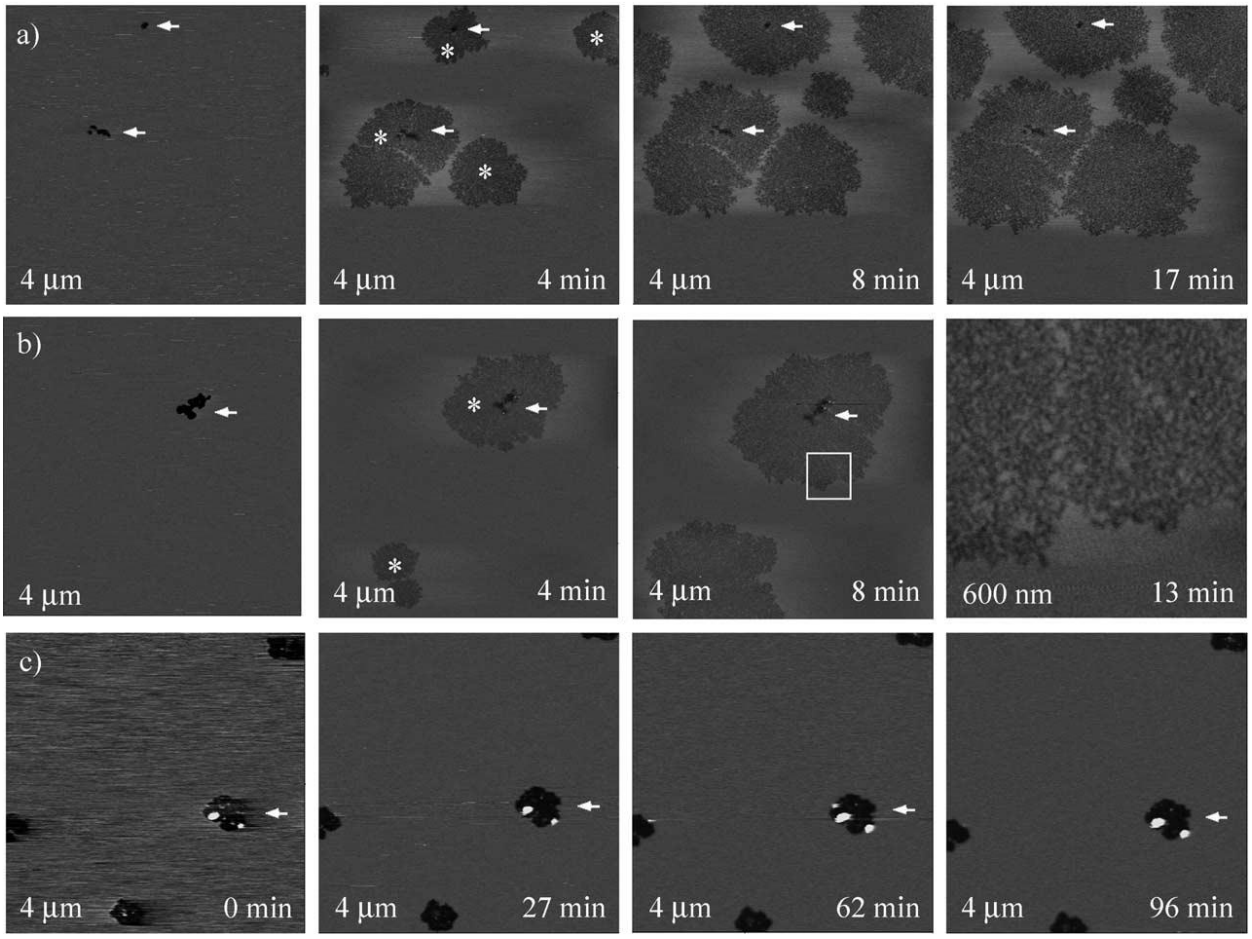
Title of Figure 4. Time-lapse AFM images of amylin fibrils growing on mica.

Legend to Figure 4: (a) AFM image of protofibrils formed after 9.5 hours. The bidirectional growth of protofibrils in the selected area is shown in the panel of four images at the bottom of (a). (b) Protofibrils can be seen forming higher order fibrils over time in a different experiment. Arrows indicates where one protofibril appears to grow from a higher order fibril. Reprinted from Goldsbury et al., copyright 1999, with permission from Elsevier (Goldsbury et al. 1999).



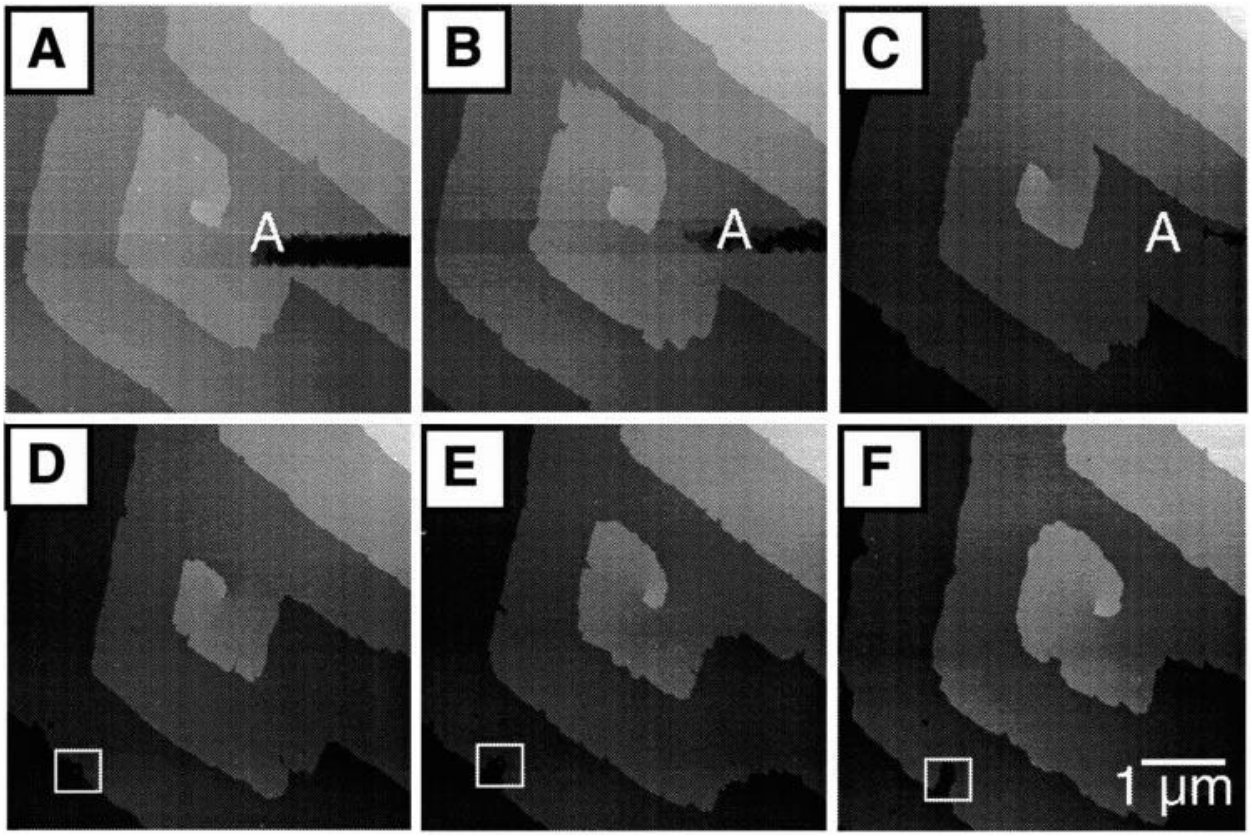
Title of Figure 5. High resolution FM-AFM images of an amylin amyloid fibril.

Legend to Figure 5: (a) 10 x 10 nm and (b) 5 x 5 nm scans of an amylin fibril on a mica substrate in PBS. The arrow in (a) indicates the location of the scan in (b). (c) Cross-section line profile plot along the line A-B in (b). (d) A quadratic line-by-line flattened image of (b). (e) Cross-section line profile plot along the line C-D in (d), which corresponds to A-B in (b). (f) Model showing the observed beta strand structure with 0.47 nm repeats. Reprinted from Fukuma et al., copyright 2008, with permission from IOP Publishing Ltd. (Fukuma et al. 2008).



Title of Figure 6. Time-lapse AFM images of a protein-membrane interaction.

Legend to Figure 6: AFM images taken after the introduction of (a) 10 mM human amylin, (b) 10 mM rat amylin, and (c) 10 mM rat amylin with 300 mM Congo red onto a palmitoyl oleoyl phosphatidylcholine/palmitoyl oleoyl phosphatidylglycerol (3:1) lipid bilayer. Defects present in the original layer are indicated by arrows. Lipid bilayer disruption is indicated by stars. The rightmost panel in (b) shows a close-up of the membrane being disrupted. Reprinted from Green et al., copyright 2004, with permission from Elsevier (Green et al. 2004).



Title of Figure 7. Time-lapse images of an insulin crystal.

Legend to Figure 7: Images were recorded at (a) 0, (b) 3605, (c) 7210, (d) 10,815, (e) 18,025, and (f) 40,590 seconds. A region damaged by the AFM tip (and subsequently repaired) is marked 'A.' In (d) a protein aggregate is indicated by a white box, which persists as a defect in the growing crystal. Reprinted from Yip et al., copyright 1998, with permission from Elsevier (Yip et al. 1998a).

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