We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

13

Single-Molecule Force Microscopy: A Potential Tool for the Mapping of Polysaccharides in Plant Cell Walls

Julian C. Thimm[1], Laurence D. Melton[2] and David J. Burritt[1]
[1]*The Department of Botany, The University of Otago, Dunedin*
[2]*Food Science Programmes, School of Chemistry Sciences, University of Auckland, Auckland*
New Zealand

1. Introduction

Plant cells are surrounded by a polysaccharide-rich cell wall that, as well as being a supporting structure (O'Neill & York, 2003), plays important roles in plant growth and development, and in the protection of plants from both biotic and abiotic stresses (Bowles, 1990). Plant cell walls are also of global economic importance, with the cell walls of food crops being of great nutritional value, while those of agricultural crops are important as a renewable resource for the textile and building industries, and increasingly as a sustainable source of fuel (O'Neill & York, 2003).

2. The plant cell wall

2.1 Plant cell wall structure

The primary plant cell wall is a three-dimensional assembly of the polysaccharides, cellulose, pectin and hemicelluloses together with water, minerals and some structural glycoproteins. Atomic force microscopy (AFM) of *Arabidopsis thaliana* leaf cell walls showed the cellulose exists as microfibrils (Davies & Harris, 2003). AFM of living celery parenchyma tissue showed the cellulose microfibrils exist in highly ordered parallel array (Thimm et al., 2000). While AFM on isolated cell walls from celery and cucumber hypocotyls that were kept hydrated showed the cellulose microfibrils were undulating in a roughly parallel manner (Thimm et al., 2009; Marga et al., 2005). Moreover, each cellulose microfibril is surrounded by matrix material (presumably pectin and hemicelluloses) that keeps the celluloses uniformly spaced apart (Marga et al., 2005; Thimm et al., 2000; Thimm et al., 2009). Small angle X-ray scattering of hydrated celery collenchyma cell walls also showed uniform spacing of cellulose microfibrils (Kennedy et al., 2007). Solid-state ^{13}C nuclear magnetic resonance spectroscopy (NMR) indicated that in mung bean hypocotyls less than 10% of the surface of cellulose microfibrils has xyloglucan adhering to it (Bootten et al., 2004) and a recent three-dimensional solid-state NMR study of *Arabidopsis thaliana* cell walls supported this finding and also showed somewhat more pectin than xyloglucan adhered to cellulose (Dick-Perez et al., 2011). Indeed, Zykwinska et al. (2007) have previously shown

arabinan and galactan side chains of pectin are bound to cellulose. This leads to two possible models for the primary cell wall. In the first cellulose microfibrils are held apart at a uniform distance by a limited number of xyloglucan crosslinks that are reinforced by more indirect crosslinks via the pectin matrix (Bootten et al., 2004; O'Neill & York, 2003). It is pertinent to note that xyloglucan has been found covalently linked to pectin in cell walls (Popper & Fry, 2008). In the alternative model the cellulose micofibrils are embedded in a matrix of pectin and hemicelluloses some of which adhere strongly to the cellulose but others interact with those bound to cellulose and the interactions diminish with the distance from cellulose (Cosgrove, 2001; Talbott & Ray, 1992). In other words a layer of other cell wall polysaccharides, of decreasing density, surrounds each cellulose rod and there is no direct cross-linking between cellulose microfibrils.

However, it is important to note the structures of the primary plant cell walls detailed above are just models, developed from a wealth of information provided by studies that have conducted chemical analyses of isolated cell walls or independently imaged isolated, or more rarely intact cell walls (Thimm et al., 2009). Hence cell wall architecture and chemistry have yet to be fully reconciled.

The chemical composition of plant cell walls is usually studied by first isolating cell walls from the plant tissues to be analysed, and then sequential extraction techniques are used to fractionate the main polysaccharide groups that make up the cell wall (Thimm et al. 2009). While isolated cell wall polysaccharides can be analysed using chemical methods or by cytochemical staining to visualise the polysaccharide types and gather information about their quantity and location, they do not give a true picture of the cell wall in its undisturbed state (Carpita et al., 1996; Thimm et al., 2002; Vian & Roland, 1991).

2.2 Imaging the plant cell wall of higher plants

While imaging techniques such as transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM) and contact mode atomic force microscopy (AFM) can provide valuable information on the appearance, arrangement and structural dimensions of cell wall components (Hansma et al., 1997; Li, 1999; Thimm et al., 2002; Thimm et al., 2009), only limited chemical information can be obtained. For example, when using AFM, the contrast mechanism used to obtain an image is the force between the tip and the sample, and many samples produce the same force, thus it is difficult to reliably determine the chemical groups that are imaged. However, single-molecule force spectroscopy (SMFS), a technique where AFM tips are modified by the addition of specific molecules has the potential to provide both structural and chemical information (Mueller et al., 2009a & 2009b).

3. Atomic force microscopy as a tool for studying plant cell walls

3.1 Contact mode AFM

Contact mode AFM is a non-destructive imaging technique by which images with nanometer resolution can be obtained, and is therefore ideal for studying cell wall structure. In this mode a sharp stylus or tip is scanned across the surface of a sample and the force between the tip and sample is measured at a series of points. A colour or grey scale is assigned to each force, and then a map of colours displays an ``image'' of the surface (Thimm et al., 2000). This form of AFM has been used to examine isolated cell wall material

from plants including apple, water chestnut, potato, carrot (Kirby et al., 1996a & 1996b) cotton (Pesacreta et al., 1997) and celery (Thimm et al., 2009), and in the "intact walls" of celery parenchyma cells (Thimm et al., 2002) and single *Zinnia elegans* tracheary elements (Lacayo et al., 2010). For a recent review of the application of AFM and other scanning probe microscopy techniques potentially useful for the study of plant cell walls see Yarbrough et al. (2009). While providing useful structural information this technique does not provide any chemical information regarding the structures observed.

3.2 Chemical and single-molecule AFM

Ducker et al. (1992) demonstrated that the forces between colloid particles could be measured by attaching a colloid particle to the end of an AFM cantilever. In biological systems, this approach has been adapted to estimate bond strength between different biological molecules such as ligands and receptors (You & Yu, 1999), antigen antibody binding (Nyquist et al., 2000), receptor binding (Raiteri et al., 1999), and the molecular assembly of biomolecules (Kossek et al., 1998, Perrin et al., 1999).

The principle of sensor force measurement involves attaching one type of molecule to the AFM tip and binding another molecule to a surface. The tip is moved towards the surface until the molecules interact. This has been demonstrated for biotin-streptavidin (Florin et al., 1994), avidin-biotin (Moy et al., 1994), for cell-cell interactions (Antonik et al., 1997), cell-adhesion proteoglycans (Dammer et al., 1995; Misevicz, 1999) and antigen-antibody systems (Dammer et al., 1996; Hinterdorfer et al., 1996). This technique can also be used to gain chemical information from the surfaces of structural components isolated from cells or from the surfaces of living cells (Mueller et al. 2009a & 2009b). For example, Gad et al. (1997) used gold-coated tips functionalized with concanavalin A to map the cell wall polysaccharides of living microbial cells.

Single-molecule force sensors can also be used to resolve topographical information (Muller et al., 2009a & 2009b). Force mapping is a force sensing technique where a small area is raster force probed. Each force curve can potentially be identified and a force map generated, showing for example the distribution of cell wall polysaccharides as mentioned above. When using single-molecule force microscopy, the probe is not in physical contact with the surface, but instead uses the attractive/repulsive part of the force curve, whereby the cantilever is purely force driven. We used a variant of this technique, with sugars attached to the tip of the cantilever, in an attempt to map the distribution of polysaccharides in the parenchyma cell walls of celery epidermal peels, produced as described in Thimm et al. (2000). In the following sections we present some preliminary findings regarding the development of sensors to both image and to determine the chemical characteristics of celery parenchyma cell walls.

4. A system for single-molecule force microscopy of celery (*Apium graveolens* L.) parenchyma cell walls using glycoside probes

4.1 The celery cell wall

Thimm et al. (2002) isolated the primary walls of celery parenchyma cells and their polysaccharide components were characterized by glycosyl linkage analysis, cross-polarization magic-angle spinning solid-state ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C

NMR) and X-ray diffraction. The cell walls consisted of mainly cellulose (43 mol%) and pectic polysaccharides (51 mol%), made up of rhamnogalacturonan (28 mol%), arabinan (12 mol%) and galactan (11 mol%), with only smalls amounts of xyloglucan (2 mol%) and xylan (2 mol%) detected in the cell walls. Solid-state ^{13}C NMR signals were consistent with the constituents identified by glycosyl linkage analysis and allowed the walls to be divided into three domains, based on the rigidity of the polymers. Cellulose (rigid) and rhamnogalacturonan (semi-mobile) polymers responded to the CP/MAS ^{13}C NMR pulse sequence and were distinguished by differences in proton spin relaxation time constants. The arabinans, the most mobile polymers, responded to single-pulse excitation (SPE), but not CP/MAS ^{13}C NMR. From solid-state ^{13}C NMR of the cell walls the diameter of the crystalline cellulose microfibrils was determined to be approximately 3 nm while X-ray diffraction of the cell walls gave a value for the diameter of approximately 2 nm.

4.2 Sensor design

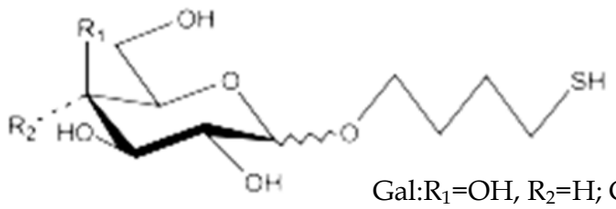
Glucose and galactose were chosen as sensor molecules as cellulose is a polymer of glucose and is one of the main structural components in the celery cell wall, while galactose is an important component of celery pectins (Thimm et al., 2002). The two main types of probes used in conventional AFM experiments are either silicon-nitride (pyramidal) or silicon (ultralever). Sugar groups can be covalently attached directly to the silanol or silolamine surface groups on commercial tips, or the commercial tip can be coated in a thin film that is convenient for further reactions. Gold is a useful film because a broad spectrum of groups can be attached to gold via thiol linkages. Thiols are well known to adsorb under mild conditions onto gold surfaces (Biebuyck & Whitesides, 1994; Fritz et al., 1996; Liu et al., 1994, Nyquist et al., 2000) and thiol adsorption is strong enough to use sugar-thiols as sensors, for these reasons we chose to use gold-coated tips.

Most interactions in biological systems depend on the orientation of the molecules. Therefore, forces between the sensor and the surface can only be detected if the sensor is able to align itself unhindered relative to the surface molecule. A carbon spacer chain was introduced to ensure that the sensor molecule had enough flexibility to sample different orientations relative to the sample. The chain-length of the spacer between the sugar ring-system and the probe determines its flexibility or ability to rotate freely, and a spacer of C4-carbons between the interacting sugar ring and the thiol-group was found to be sufficient for probing plant cell wall extracts and surfaces (Thimm, 2000).

4.3 Synthesis of glycoside-sensors and attachment of glycoside-sensors to gold-coated cantilevers

The synthetic approach for the derivatisation of thiobutyl-glycosides with a C4-spacer (butyl-spacer) can be found in Thimm (2000) and the probes used are shown in Figure 1.

To avoid contamination of the gold-coated AFM cantilevers, all sensor attachment procedures were conducted in a laminar flow cabinet, in 9 cm polystyrene petri dishes. A glass-slide was cut to fit inside a petri dish and 30 min prior to sensor attachment, distilled ethanol was poured around the glass-slide and the dish was covered to saturate the atmosphere with ethanol vapour. The cantilevers were placed onto the glass-slide and 3-4 drops of a dilute ethanolic solution (1 to 5 mmol) of the thio-glycosides was placed onto the gold-coated cantilever and the petri dish was covered. If the sample appeared to be drying



Gal:R_1=OH, R_2=H; Glc:R_1=H, R_2=OH

Fig. 1. The probes used in the experiments detailed below

out within the incubation time, a drop of distilled ethanol was added. The surfaces were then carefully washed with ethanol and then deionised water, and stored wet. Force experiments were conducted where glycoside-sensors were used to probe gold-coated surfaces, to which glycosides were attached, to the determine if the sensors were sufficiently coated with thio-glycosides and to determine the functionality of the system (Thimm, 2000).

5. Force mapping celery cell wall fractions

5.1 Cell wall fractionation

As discussed in section 3 plant cell walls can be studied by selectively fractionating the polysaccharide groups that make up the wall, using sequential extractions, so that each fraction can be studied in more detail (Melton & Smith, 2005). Water-soluble pectins are extracted with boiling water or cold HEPES buffer, while less-methylated pectins, held in the wall with calcium bridges, are solubilised with calcium chelating agents such cyclohexane-trans-1,2-diaminetetraacetic acid (CDTA). Esterified pectins, and some hemicelluloses are extractable with weak alkali (sodium carbonate, Na_2CO_3). Hemicelluloses and some branched pectins are extracted with 1M KOH, while 4M KOH is used to extract more hemicelluloses, including xylogucans, and additional branched pectins (Siddiqui, 1989a and 1989b). The insoluble final residue is largely cellulose, with any remaining hemicelluloses and some complex pectins (Percy et al., 1997, Redgwell et al., 1997; Thimm et al., 2009).

We used force mode AFM, using the glycoside-sensors detailed in the last section, to probe the cell wall components isolated/extracted from celery parenchyma cells as described by Thimm et al. (2009). The components probed were the final insoluble residue largely composed of cellulose, water-soluble pectins (referred to as the HEPES fraction) and the esterified pectins, and some hemiceluloses (referred to as the carbonate fraction). Briefly a dilute (1-4 mg/mL) 20 % ethanolic suspension of each component was adsorbed onto freshly cleaved mica and allowed to dry in air for up to 20 min. To avoid floating particles the sample was careful rinsed with 1-2 mL dionized water (Thimm et al., 2009). The cell wall components were then probed using glycoside-sensors and force curves generated for the cell wall components detailed above.

5.2 The final insoluble residue (cellulose)

To determine if the force curves associated with the final residue were largely due to the chemical properties of cellulose, a standard (Whatman cellulose, CF11) was probed and the force curves compared to those of the insoluble final residue. The force curves for both the

CF11 and the final insoluble residues were similar (Figure 2). The force curves showed jumps starting at 8 to 8.3 nm distance and less defined jumps at 3 to 3.1 nm and 2 to 2.3 nm distance. The force curves generated when the final insoluble residue was probed with either a galactoside or glucoside sensor showed no clear differences (Figure 3).

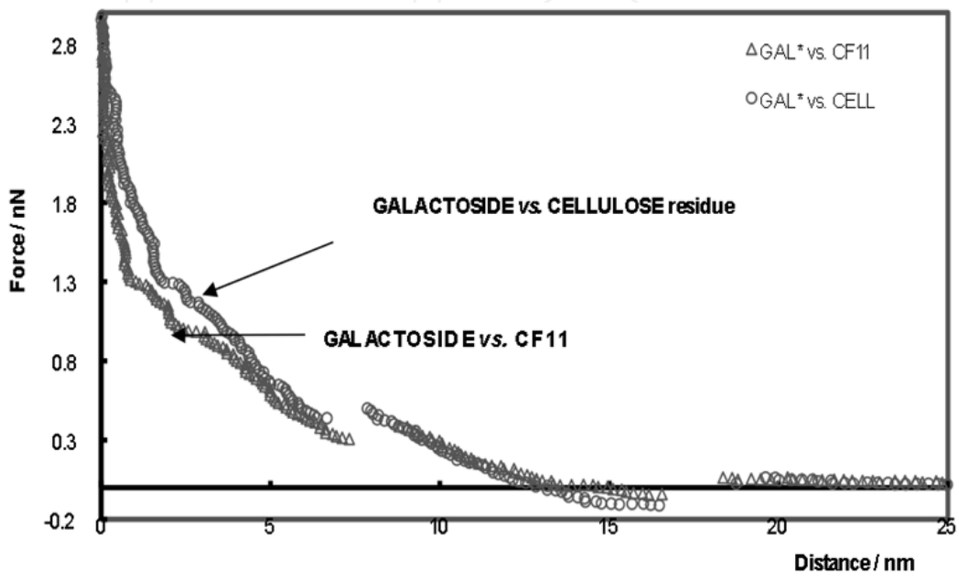


Fig. 2. Force-distance plots for a galactoside-sensor vs. the final cell wall residue (largely cellulose) and a galactoside-sensor vs. cellulose CF11.

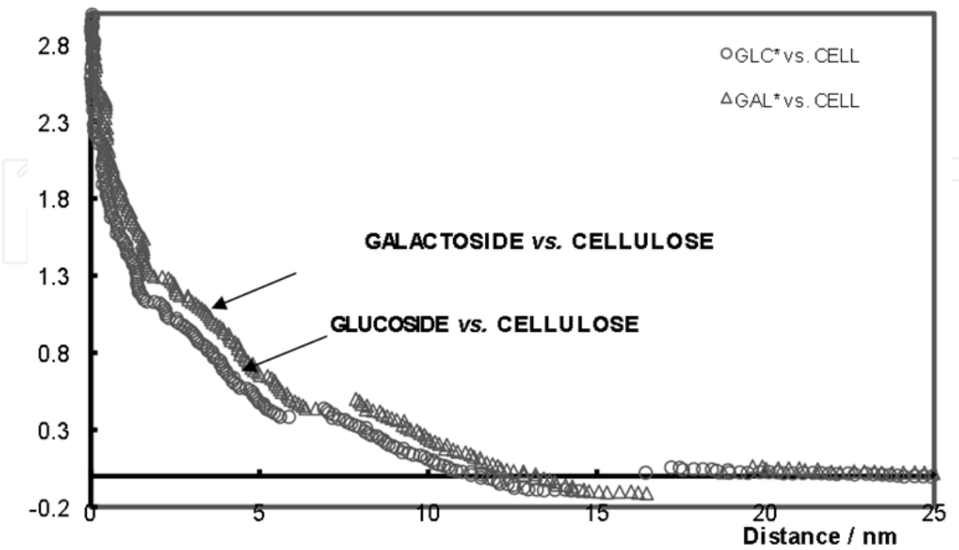


Fig. 3. Force-distance plots for galactoside- and glucoside-sensor vs. the final cell wall residue (largely cellulose).

5.3 Water-soluble pectins (the HEPES fraction)

Figure 4 shows a force-distance plot for a glucoside-sensor vs. the HEPES fraction. Jumps are observed at 49 ± 2 , 42 ± 2 (weak), 37.5 ± 2, 23.3 ± 1, 13.5 ± 1, 6.1 ± 0.5 (weak), 3.1 ± 0.1 and 1.7 nm ± 0.2.

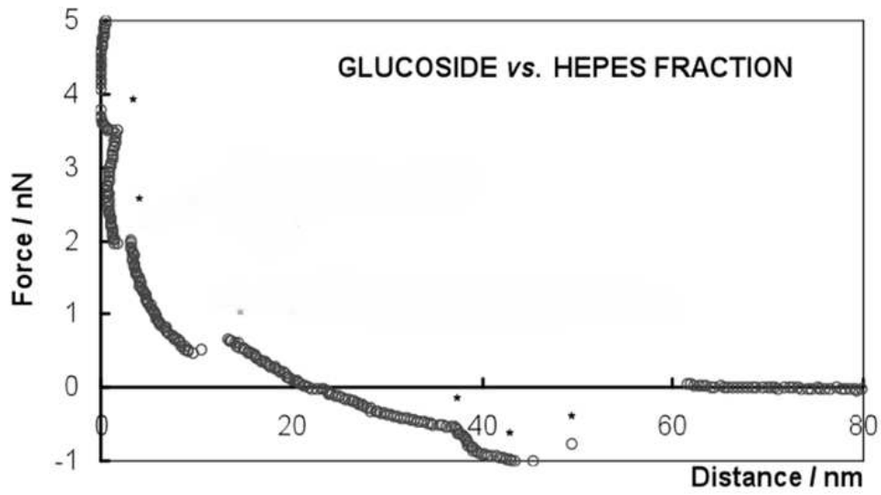


Fig. 4. A force-distance plot for a glucoside-sensor vs. the HEPES fraction. No differences were observed between the glucoside- and galactoside-sensors. Jumps are indicated by an *.

As with the final cell wall residue (cellulose) the glucoside- and galactoside-sensors showed only minor differences in their respective force curves and so could not be used as individual sensors to distinguish galactoses from glucoses and vice versa.

5.4 Esterified pectins and some hemicelluloses (the carbonate fraction)

Figure 5 shows a force-distance plot for a glucoside-sensor vs. the carbonate fraction. Two distinct jumps were observed at 16.5 nm ± 1 (with a displacement force of 0.2 nM ± 0.1) and at 2.9-3.2 nm (0.1 nN ± 0.05). Two weak repulsions at 5.4 ± 0.1 and 9.2 nm ± 0.7 were also observed, but these were often not well resolved.

5.5 Mixture of fractions

Force mapping experiments were also conducted on mixtures of the three cell wall components in an attempt to simulate an *in situ* system. This experiment attempted to determine if cell wall polysaccharides could be identified, by their force curves, in a complex mixture and if the results could then be used to generate a polysaccharide distribution map. A mixture of the three cell wall components (1:1:1 w/w) were adsorbed onto a mica sheet and the sheet was force mapped using a glucoside-sensor. Figure 6 shows a polysaccharide distribution map of such a mixture, showing the distribution of the three cell wall components, identified based on their force curves.

For one sample of mixed components, 242 force curves were generated. Of these, 78 counts (32 %) were for cellulose, possibly indicating that cellulose could readily be detected on the

surface, possibly as a result of swollen microfibrils that are larger and hence were more easily detected that the other cell wall components.

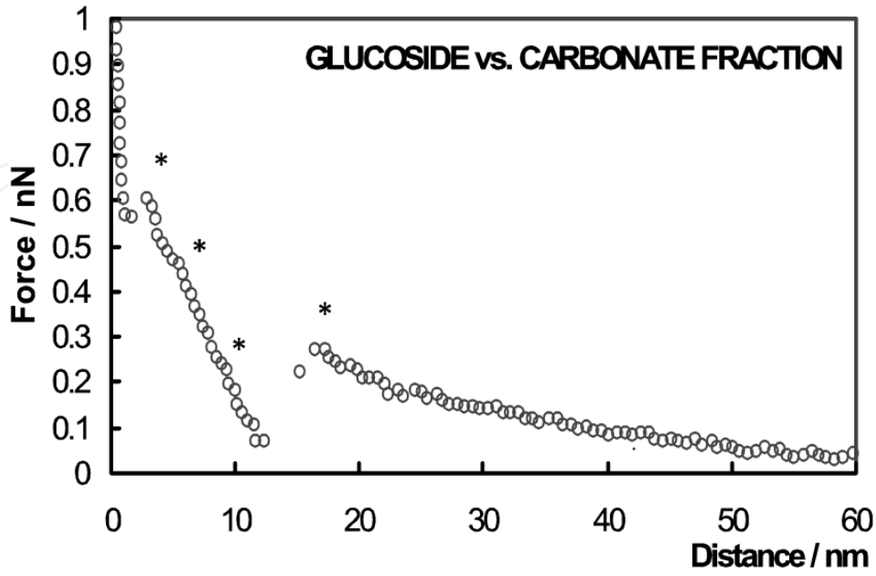


Fig. 5. A representative force-distance plot for a glucoside-sensor vs. the carbonate fraction. Jumps are indicated by an *.

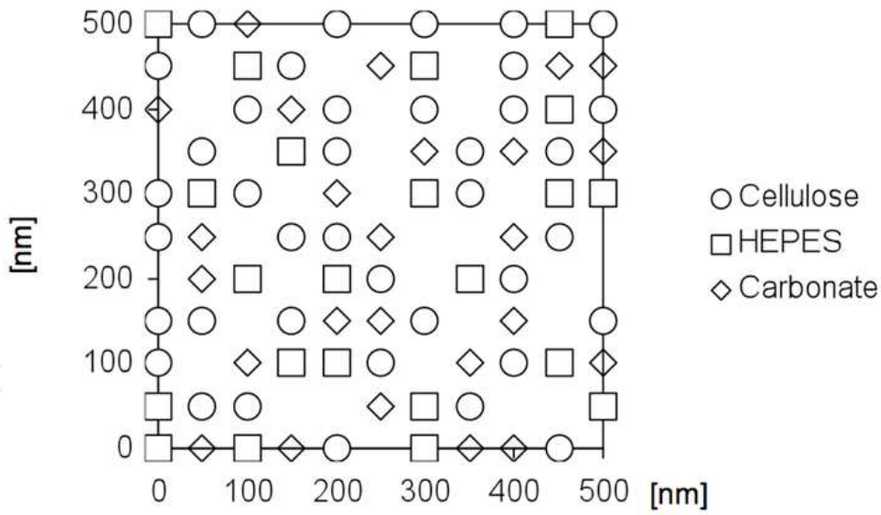


Fig. 6. A force curve distribution map of three cell wall components adsorbed onto a mica sheet and force mapped using glucoside-sensor.

The HEPES fraction was detected 44 times (18 %). This result was unexpectedly low and may have indicated preferred adsorption of the HEPES polysaccharides to the mica surface. Mica (muscovite) is a potassium aluminium silicate hydroxide fluoride that exposes silicate oxygen atoms at the surface, resulting in a negative charge in water. This charge might induce the preferred adsorption of particular polysaccharides from the polysaccharide mixture. Once the first species is adsorbed onto mica, their properties might drive the

aggregation of the next layer. It is possible that the HEPES fraction adheres more quickly to the mica than the other cell wall components. The other cell wall components could then form a second or third layer on top of the HEPES fraction and thus it would be less accessible to the sensor, which probes only the surface.

The carbonate fraction was only detected slightly more frequently (21 %) than the HEPES fraction. This pectic material might show a similar behaviour as the HEPES fraction and hence compete for mica surface space. Adsorption to mica is possibly through the formation of electrostatic bonds with the uronic acids of the HEPES fraction. Greater branching and methyl-esterification of the carbonate fraction could result in a reduced ability to compete for mica, even though the sodium carbonate extraction might have caused saponification of methyl-esters to some degree. Another possible explanation might be that the three cell wall components aggregate in solution prior to adsorption to the mica.

A large number (70 counts (29 %) of 242 force curves) of force curves could not be identified. One factor contributing to this was the surface of the polysaccharide mixture seemed to be very rough for force experiments. During raster scanning, forces were probed in regions consisting of gaps or raised regions of the surface. Another explanation might be that forces that could not be assigned are a direct result of intermolecular aggregation of the three cell wall components in solution, resulting in new unidentified force profiles. The distribution maps for the three cell wall components showed an even distribution covering most of the scanned region. No accumulation of individual cell wall components at particular areas was observed.

6. Force mapping the celery cell wall *in situ*

6.1 Imaging using force mode

Figure 7 shows an AFM deflection image of a celery epidermal peel *in situ*, captured in force mode, using a glucoside sensor. Force mode was used to image the cell in order to prevent or minimize contamination of the sensor by reducing physical contact with the surface. The force mode images of the cell wall of celery parenchyma epidermal peels are not as sharp or defined as previously published contact mode images (Thimm et al., 2000).

6.2 Force mapping the surface *in situ*

Prior to force mapping the cell wall, a force mode image was captured and was used to guide the probe (reading x, y coordinates in nm) when force mapping the surface *in situ*. For the image shown in Figure 7, 126 force curves were generated, from different points on the sample and the structural identity of each point was determined by comparison to the forces curves generated from the isolated cell wall components. For each component of the wall, an example of a force curve from the *in situ* experiments is shown below, compared to the isolated cell wall components, cellulose (Figure 8), HEPES (Figure 9) and carbonate (Figure 10).

Of the 126 force curves generated *in situ* using the glucoside-sensor, 53 (42 %) could be attributed to cellulose, 9 (7 %) could be attributed to HEPES fraction polysaccharides and 13 (10 %) could be attributed to carbonate fraction polysaccharides. Of the 126 curves, 51 (41 %) could not be identified. The large number of forces that could not be assigned could be due to the presence of non-polysaccharide material, to aggregated polysaccharides or to

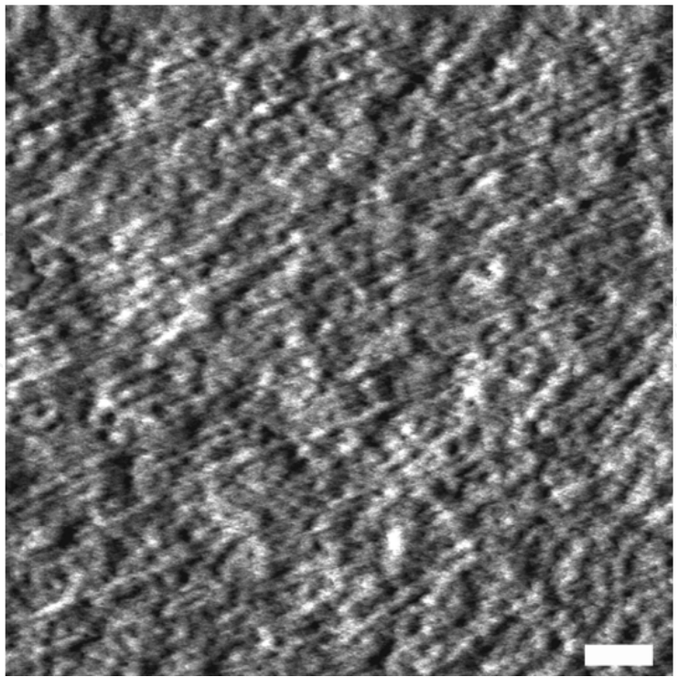


Fig. 7. AFM force mode deflection image of celery epidermal peels in situ captured using a glucoside sensor (scan size 2 µm, scale bar 200 nm, height scale 5 nm)

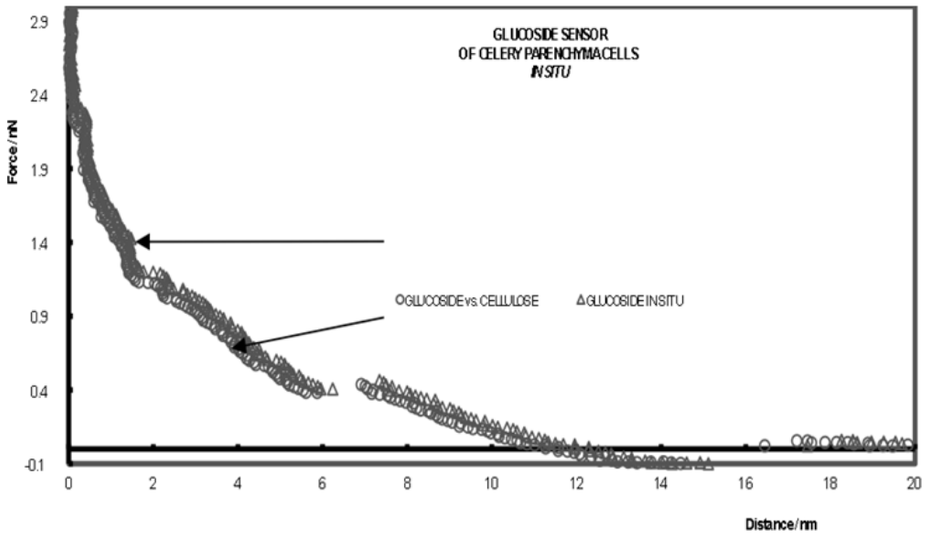


Fig. 8. Representative force-distance plots, using a glucoside-sensor, showing two over laid force curves. One for celery parenchyma cells *in situ* and the second for the cell wall final residue (mainly cellulose).

polysaccharide components of the cell wall for which no reference force curves were available. Most of the force curves that could not be identified showed a strong exponential repulsive behaviour, which could be due to the presence of high molecular weight polymers or aggregates.

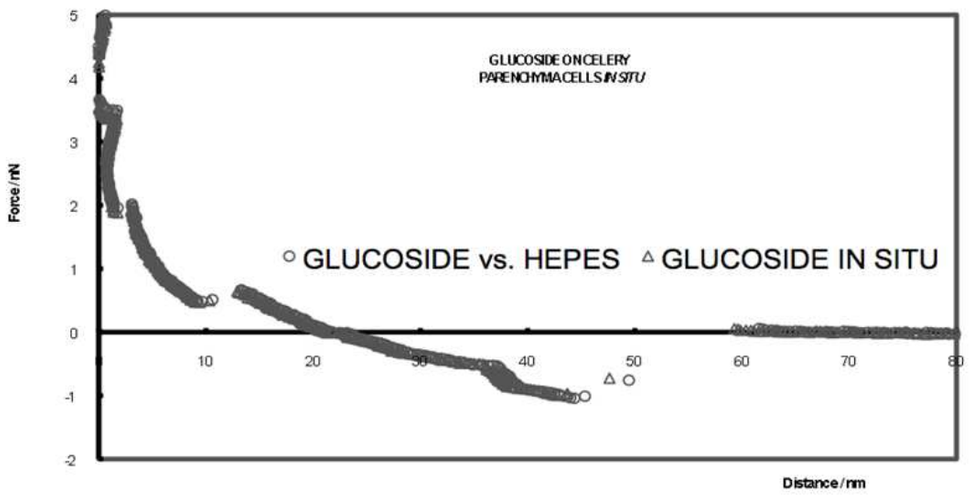


Fig. 9. Representative force-distance plots, using a glucoside sensor, showing two over laid force curves. One for celery parenchyma cells *in situ* and the second for HEPES fraction.

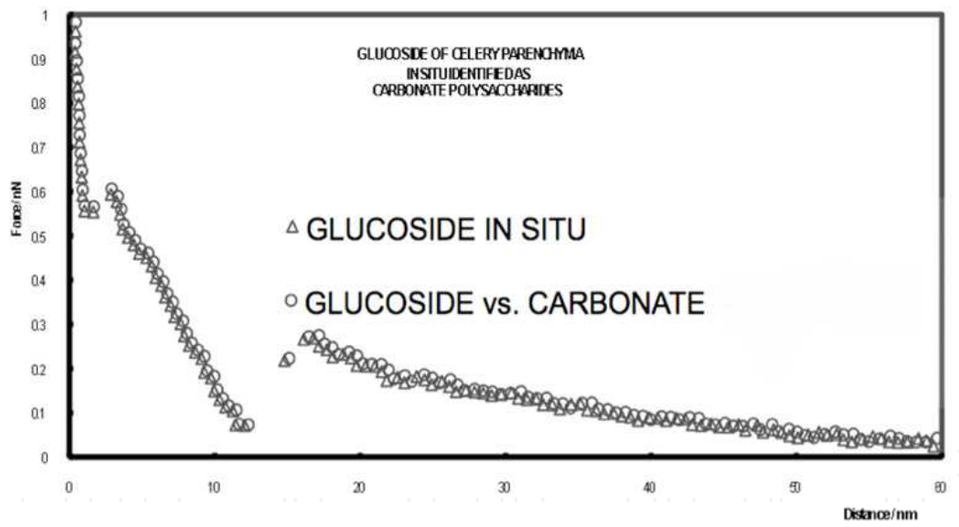


Fig. 10. Representative force-distance plots, using a glucoside-sensor, showing two over laid force curves. One for celery parenchyma cells *in situ* and the second for the carbonate fraction.

By overlaying data points of the force maps for each identified cell wall component, with the force mode AFM deflection image in Figure 7, an image of the wall *in situ* with some chemical information could be generated (Figure 11). Force curves identified as belong to the final cell wall residue did appear to be localised more frequently on the raised microfibril like structures clearly seen in Figure 11. However, no distinct pattern of localisation of the three cell wall components to any particular area of the image was consistently observed.

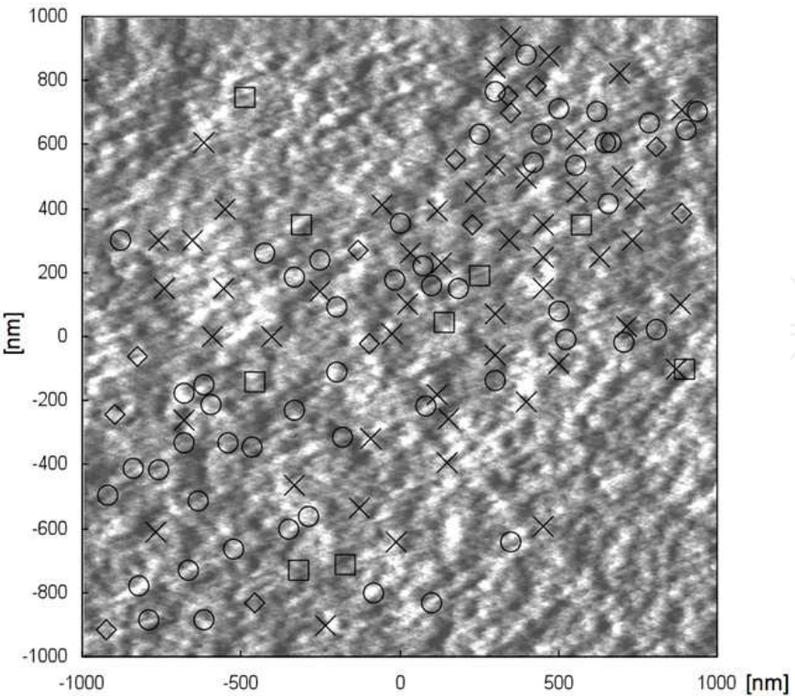


Fig. 11. A map of a celery epidermal cell wall *in situ*, showing the cell wall component distribution (cellulose, circle; HEPES, square; carbonate, diamond; unassigned forces, X).

7. Conclusions

The use of force curves generated by single molecule sensors is potentially a useful way that information about the chemical nature, the strength of interactions, and possibly the molecular arrangement of complex biological structures can be deduced (Gad et al., 1997; Hansma et al., 1997; Li et al., 1998; Li et al., 1999; Marzalek et al., 1998; Osada et al., 1999; Okabe et al., 2000; Rief et al., 1997). To date a few attempts have been made to force probe intact plant cell walls *in situ*. The approach taken in our investigation demonstrates the potential for using single molecule force microscopy to study plant cell wall chemistry and structure and opens up a variety of possible uses, depending on the type of sensor chosen. By probing, using the glucoside-sensors, the three celery cell wall components (HEPES fraction, carbonate fraction and the final residue) in isolation and a mixture of them, we have shown that individual cell wall components can be distinguished in a complex mixture.

When force mode imaging, using glucoside-sensors, was combined with force curve identification, it was possible to map the distribution of cell wall components in celery epidermal peels *in situ*. However, while a cell wall component distribution map of celery epidermal peels *in situ* was obtained, showing the chemical information overlaid over a topographical AFM force mode image, the HEPES and carbonate fraction polysaccharides did not show any regular or ordered distribution. Only force curves identified as belong to the final cell wall residues showed some localisation, appearing more frequently on the raised microfibril-like structures.

For celery, the chemical resolution of the force map was limited to the number of calibration standards produced. We used only three polysaccharide extracts (HEPES fraction, carbonate

fraction and the final cellulose residue). The quality and/or purity of the fractions used as standards is unknown, because it is not known how many different types of polysaccharides are present in, for example, the HEPES or carbonate fraction. More sophisticated extraction and purification methods could be used to produce cell wall standards to generate force curves that could be interpreted in more detail.

By increasing the number of calibration standards a larger number of forces could be identified. Ideally polysaccharides that are highly purified or synthesised, to give molecules of known composition, molecular arrangement and linkage type (e.g. xyloglucan, xylan) could be used. A different approach might be to mimic polysaccharide structures in the plant cell wall by synthesising identified structural elements of, for example, polysaccharide sidechains of pectin (galactans and arabinans). Their conformation, strength and fingerprint forces could then be studied systematically.

In the present study, a number of forces could not be identified. One explanation for this was the lack of calibration standards, but another important factor might be the interaction of polysaccharides in solution and *in situ.* Aggregation of polysaccharides has been observed for xyloglucan and cellulose as well as pectin sidechains and cellulose (Pauly et al., 1999; Whitney et al. 1995 & 1999; Zykwinska et al., 2007) and this could influence the force curve observed. Force measurements of calibration standards (e.g. hemicelluloses such as xyloglucan and xylan, pectins such as homogalacturonan, arabinan, galactan or arabinogalactan) need to be made of polysaccharides mixed in solution prior to adsorption on to a mica surface. In addition, further experiments need to be conducted to determine what influences polysaccharide aggregation might have on the observed force curves.

8. References

Antonik, M.D.; D'Costa, N.P.; Hoh J.H. (1997). A biosensor based on micromechanical interrogation of living cells. *IEEE Engineering in Medicine & Biology Society*. Vol.16, 66-72.

Biebuyck, H.A; Whitesides, G.M. (1994). Self organization of organic liquids on patterned self-assembled monolayers of alkanethiolates on gold. *Langmuir,* Vol.10, 2790-2793.

Bootten, T.J.; Harris; P.J., Melton, L.D.; Newman, R.H. (2004). Solid-state ^{13}C-NMR spectroscopy shows that xyloglucan in the primary cell walls of mung beans (*Vigna radiata* L.) occur in different domains: a new model for xyloglucan-cellulose interactions in the cell wall. *Journal of Experimental Botany*, Vol.55, 571-583.

Bowles, D.J. (1990). Defence-related proteins in higher plants. *Annual Review of Biochemistry*, Vol.59, 873-907

Carpita, N.C.; McCann, M.; Griffing, L.R.; (1996) The plant extracellular matrix: news from the cell's frontier. *Plant Cell*, Vol 8, 1451-1463.

Cosgrove D.J. (2001). Wall structure and wall loosening. A look backwards and forwards. *Plant Physiology*, Vol.125, 131-134.

Dammer, U.; Hegner, M.; Anselmetti, D.; Wagner, P.; Dreier, M.; Huber, W.; Guntherodt, H.J. (1996). Specific antigen and antibody interactions measured by atomic force microscopy. *Biophysical Journal,* Vol.70, 2437-2441.

Dammer, U.; Popescu, O.; Wagner, P.; Anselmetti, D.; Guentherodt, H.J.; Misevic, G.N. (1995). Binding strength between cell adhesion proteoglycans measured by force microscopy. *Science*, 267, Vol.1173-1175.

Davies, L.M.; Harris, P.J. (2003) Atomic force microscopy of microfibrils in primary cell walls. *Planta,* Vol.217, 283-289.

Dick-Perez, M.; Zhang, Y.; Hayes, J.; Salazar, A.; Zabotina, O.A.; Hong, M. (2011). Structure and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. *Biochemistry*, Vol.50, 989-1000.

Ducker, W.A.; Senden, T.J.; Pashley, R.M. (1992). Measurement of forces in liquids using a force microscope. *Langmuir,* Vol.8, 1831-1836.

Florin, E.L.; Moy, V.T.; Gaub, H.E. (1994). Adhesion forces between individual ligand receptor pairs. *Science,* Vol.264, 415-417.

Fritz, M.C.; Hähner, G.; Spencer, N.D. (1996). Self-assembled hexasaccharides: surface characterisation of thiol-terminated sugars adsorbed on a gold surface. *Langmuir,* Vol.12, 6074-6082.

Gad, M.; Itoh, A.; Ikai, A. (1997) Mapping cell wall polysaccharides of living microbial cells using atomic force microscopy. *Cell Biology International,* Vol.21, 697-706.

Hansma, H. G.; Kim, K. J.; Laney, D. E.; Garcia, R. A.; Argaman, M.; Allen, M. J.; Parsons, S. M. (1997). Properties of biomolecules measured from atomic force microscope images: a review. *Journal of Structural Biology,* Vol.119, 99-108.

Hansma, H.G.; Hoh, H.J. (1994). Biomolecular imaging with the atomic force microscope. *Annual Review of Biophysics & Biomolecular Structure*, Vol.23, 115-139.

Hinterdorfer, P.; Baumgartner, W.; Gruber, H.J.; Schilcher, K.; Schindler, H. (1996). Detection and localization of individual antibody-antigen recognition events by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.93, 3477-3481.

Kennedy, C.J.; Sturcova, A.; Jarvis M.C.; Wess, T.J. (2007). Hydration effects on spacing of primary-wall cellulose microfibrils: a small angle X-ray scattering study. *Cellulose,* Vol.14, 401-408.

Kirby, A.R.; Gunning, A.P.; Morris, V.J. (1996a). Imaging polysaccharides by atomic force microscopy. *Biopolymers,* Vol.38, 355-366.

Kirby, A.R.; Gunning, A.P.; Waldron, K.W.; Morris, V.J.; Ng A. (1996b). Visualization of plant cell walls by atomic force microscopy. *Biophysical Journal*, Vol.70, 1138-1143.

Kossek, S.; Padeste, C.; Tiefenauer, L.X.; Siegenthaler, H. (1998) Localisation of individual biomolecules on sensor surfaces. *Biosensors & Bioelectronics*, Vol.13, 31-43.

Lacayo, C.I.; Malkin, A.J.; Holman, H.N.; Chen, L.A.; Ding, S-Y.; Hwang, M.S.; Thelen, M.P. (2010) Imaging Cell Wall Architecture in Single *Zinnia elegans* Tracheary Elements. *Plant Physiology*, Vol.154, 121-133.

Li, H.B.; Rief M.; Oesterhelt, F.; Gaub, H.E.; Zhang, X.; Shen, J. (1999) Single-molecule force spectroscopy on polysaccharides by AFM-nanomechanical fingerprint of alpha-(1,4)-linked polysaccharides. *Chemical Physics Letters,* Vol.305, 197-201.

Li, H.B.; Rief, M.; Oesterhelt, F.; Gaub, H.E. (1998). Single-molecule force spectroscopy on xanthan by AFM. *Advanced Materials*, Vol.10, 316-319.

Li, M.Q. (1999). Scanning probe microscopy (STM/AFM) and applications in biology. *Applied Physics (A) Materials Science and Processing,* Vol.68, 255-258.

Liu, Y.; Wu, T.; Fennell, E.D. (1994). Lateral force microscopy study on the shear properties of self assembled monolayers of dialkylammonium surfactant on mica. *Langmuir,* Vol.10, 2241-2245.

Marga, F.; Grandbois, M.; Cosgrove, D.J.; Baskin, T.I. (2005) Cell wall extension results in the coordinate separation of parallel microfibrils: evidence from scanning electron microscopy and atomic force microscopy. *Plant Journal,* Vol.43, 181-190.

Marszalek, P.E.; Oberhauser, A.F.; Pang, Y-P. Fernandez J.M. (1998). Polysaccharide elasticity governed by chair-boat transisitions of the glucopyranose ring. *Nature,* Vol.396, 661-664.

Melton, L.D.; Smith, B.G. (2005). Isolation of plant cell walls and fractionation of cell wall polysaccharides. In: Wrolstad, R. (Ed.), Handbook of Food Analytical Chemistry: Water, Proteins, Enzymes, Lipids and Carbohydrates. Wiley- Interscience, New Jersey, pp. 697-719.

Misevicz, G.N. (1999) Molecular self-recognition and adhesion via proteoglycan interactions as a pathway to multicellularity: Atomic force microscopy and color coded bead measurements in sponges. *Microscopy Research and Technique,* Vol.44, 304-309.

Morris, V.J.; Gunning, A.P.; Gunning, A.P.; Kirby, A.R.; Round, A.; Waldron, K.; Ng, A. (1997). Atomic force microscopy of plant cell walls, plant cell wall polysaccharides and gels. *International Journal of Biological Macromolecules,* Vol.21, 61-66.

Morris, V.J.; Kirby, A.R.; Gunning, A.P. (1999). Atomic force microscopy for biologists, Imperial College Press.

Moy, V.T.; Florin E.L.; Gaub H.E. (1994). Intermolecular forces and energies between ligands and receptors. *Science,* Vol.266, 257-259.

Mueller, D.J.; Helenius, J.; Alsteens, D.; Dufrene, Y.F. (2009a) Force probing surfaces of living cells to molecular resolution. *Nature Chemical Biology*, Vol.5, 383-390.

Mueller, D.J.; Krieg, M.; Alsteens, D.; Dufrene, Y.F. (2009b) New frontiers in atomic force microscopy: analyzing interactions from single-molecules to cells. *Current Opinion in Biotechnology,* Vol.20, 4-13.

Nyquist, R.M.; Eberhardt, A.S.; Silks, L.A.; Li, Z.; Yang, X.; Swanson, B.I. (2000). Characterisation of self-assembled monolayers for biosensor applications. *Langmuir,* Vol.16, 1793-1800.

O'Neill, M.A.; York,W.S. (2003). The composition and structure of plant primary cell walls. In: Rose, J. K. C. (Ed.), The Plant Cell Wall. Blackwell, Oxford. pp.1-54.

Okabe, Y.; Akiba, U.; Fujihira M. (2000). Chemical force microscopy of -CH_3 and -COOH terminal groups in mixed self-assembled monolayers by pulsed-force-mode atomic force microscopy. *Applied Surface Science,* Vol.157, 398-404.

Osada, T.; Takezawa, S.; Itoh, A.; Arakawa, H.; Ichikawa, M.; Ikai, A. (1999). The distribution of sugar chains on the vomeronasal epthelium observed with an atomic force microscope. *Chemical Senses,* Vol.24, 1-6.

Pauly, M.; Albersheim, P.; Darvill, A.; York, W.S. (1999). Molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. *Plant Journal,* Vol.20, 629-639.

Percy, A.E.; Melton, L.D.; Jameson, P.E. (1997). Xyloglucan and hemicelluloses in the cell wall during apple fruit development and ripening. *Plant Science*, Vol.125, 31-39.

Perrin, A.; Theretz, A.; Lanet, V.; Vialle, S.; Mandrand, B. (1999) Immunomagnetic concentration of antigens and detection based on a scanning force microsocpe. *Journal of Immunological Methods,* Vol.224, 77-87.

Pesacreta T.C., Carlson L.C. and Triplett BA (1997). Atomic force microscopy of cotton fiber cell wall surfaces in air and water: quantitative and qualitative aspects. *Planta*, Vol.202, 435-442.

Popper, Z.A.; Fry, S.C. (2008). Xyloglucan-pectin linkages are formed intra-protoplasmically, contribute to wall assembly, and remain stable in the cell wall. *Planta,* Vol.227, 781-794.

Raiteri R., Nelles G., Butt H.J., Knoll W. and Skladal P. (1999). Sensing of biological substances based on the bending of microfabricated cantilevers. — Sensors and Actuators B-Chemical 61: 213-217.

Redgwell, R.J.; Fischer, M.; Kendal, E.; Macrae, E.A.; (1997). Galactose loss and fruit ripening - high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta,*Vol. 203, 174-181.

Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub H.E. (1997). Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science,* Vol.275, 1295-1297.

Siddiqui, I.R., 1990a. Studies on vegetables. Investigation of water, oxalate, and sodium hydroxide soluble celery (*Apium graveolens*) polysaccharides: Celery arabinogalactan. *Journal of Agriculture & Food Chemistry,* Vol.38, 38-41.

Siddiqui, I.R., 1990b. Studies on vegetables. Investigation of water, oxalate, and sodium hydroxide soluble celery (*Apium graveolens*) polysaccharides: Pectic polysaccharides. *Journal of Agriculture & Food Chemistry,* Vol.38, 70-74.

Talbott, L.D.; Ray, P.M. (1992) Molecular size and separability features of pea wall polysaccharides. Implications for models of primary wall structure. *Plant Physiology*, Vol.92, 357-368.

Thimm, J.C. (2000) The plant cell wall architecture of celery (*Apium graveolens* L). Ph.D. Thesis, The University of Otago, Dunedin, New Zealand.

Thimm, J.C.; Burritt, D.J.; Ducker, W.A.; Melton, L.D (2009) Pectins influence microfibril aggregation in celery cell walls: An atomic force microscopy study. *Journal of Structural Biology,* Vol.168, 337-344.

Thimm, J.C.; Burritt, D.J.; Ducker, W.A.; Melton, L.D. (2000) Celery (Apium graveolens) parenchyma cell walls examined by atomic force microscopy: effect of dehydration on cellulose microfibrils. *Planta,* Vol.212, 25-32.

Thimm, J.C.; Burritt, D.J.; Sims, I.M.; Newman, R.H.; Ducker, W.A.; Melton, L.D. (2002). Celery (*Apium graveolens* L.) parenchyma cell walls: Cell walls with minimal xyloglucan. *Physiologia Plantarum,* Vol.116, 164-171.

Vian, B.; Roland, J.C. (1991). Affinodetection of the sites of formation and of the further distribution of polygalacturonans and native cellulose in growing plant-cells. *Biology of the Cell,* Vol.71, 43-55.

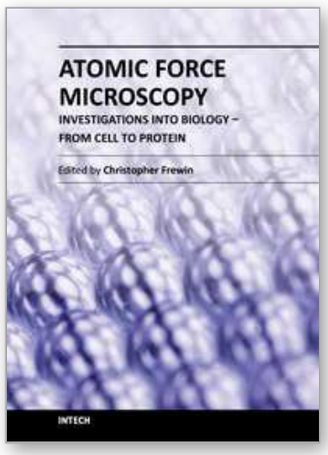
Whitney, S.; Brigham, J.; Darke, A.H.; Reid, J.S.G.; Gidley M.J. (1995). *In vitro* assembly of cellulose/xyloglucan networks: Ultrastructural and molecular aspects. *Plant Journal,* Vol.8, 491-504.

Whitney, S.E.C.; Gothard, M.G.E.; Mitchell, J.T.; Gidley, M.J. (1999). Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. *Plant Physiology,* Vol.121, 657-663.

Yarbrough, J.M.; Himmel, M.E.; Ding, S-Y. (2009). Plant cell wall characterization using scanning probe microscopy techniques. Biotechnology for Biofuels, Vol.2, 17, doi:10.1186/1754-6834-2-17.

You, H. X.; Yu, L. (1999). Atomic force microscopy imaging of living cells: progress, problems and prospects. *Methods in Cell Science,* Vol.21, 1-17.

Zykwinska, A.; Thibault, J.F.; Ralet, M.C. (2007). Organization of pectic arabinan and galactan side chains in association with cellulose microfibrils in primary cell walls and related models envisaged. *Journal of Experimental Botany,* Vol.58, 1795-1802.



Atomic Force Microscopy Investigations into Biology - From Cell to Protein

Edited by Dr. Christopher Frewin

ISBN 978-953-51-0114-7
Hard cover, 354 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

The atomic force microscope (AFM) has become one of the leading nanoscale measurement techniques for materials science since its creation in the 1980's, but has been gaining popularity in a seemingly unrelated field of science: biology. The AFM naturally lends itself to investigating the topological surfaces of biological objects, from whole cells to protein particulates, and can also be used to determine physical properties such as Young's modulus, stiffness, molecular bond strength, surface friction, and many more. One of the most important reasons for the rise of biological AFM is that you can measure materials within a physiologically relevant environment (i.e. liquids). This book is a collection of works beginning with an introduction to the AFM along with techniques and methods of sample preparation. Then the book displays current research covering subjects ranging from nano-particulates, proteins, DNA, viruses, cellular structures, and the characterization of living cells.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen