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Immunolabeling as a tool for understanding the spatial distribution of fiber wall components and their biosynthetic enzymes

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NC STATE UNIVERSITY

OUTLINE

I. Why does immunolabeling matter in nanotechnology?

II. What is immunolabeling?

III. Applications of immunolabeling

a) Revealing mechanisms of cellulose biogenesis

b) Illustrating spatial chemistry of the native raw material

c) Correlating fiber wall chemistry with nanostructure

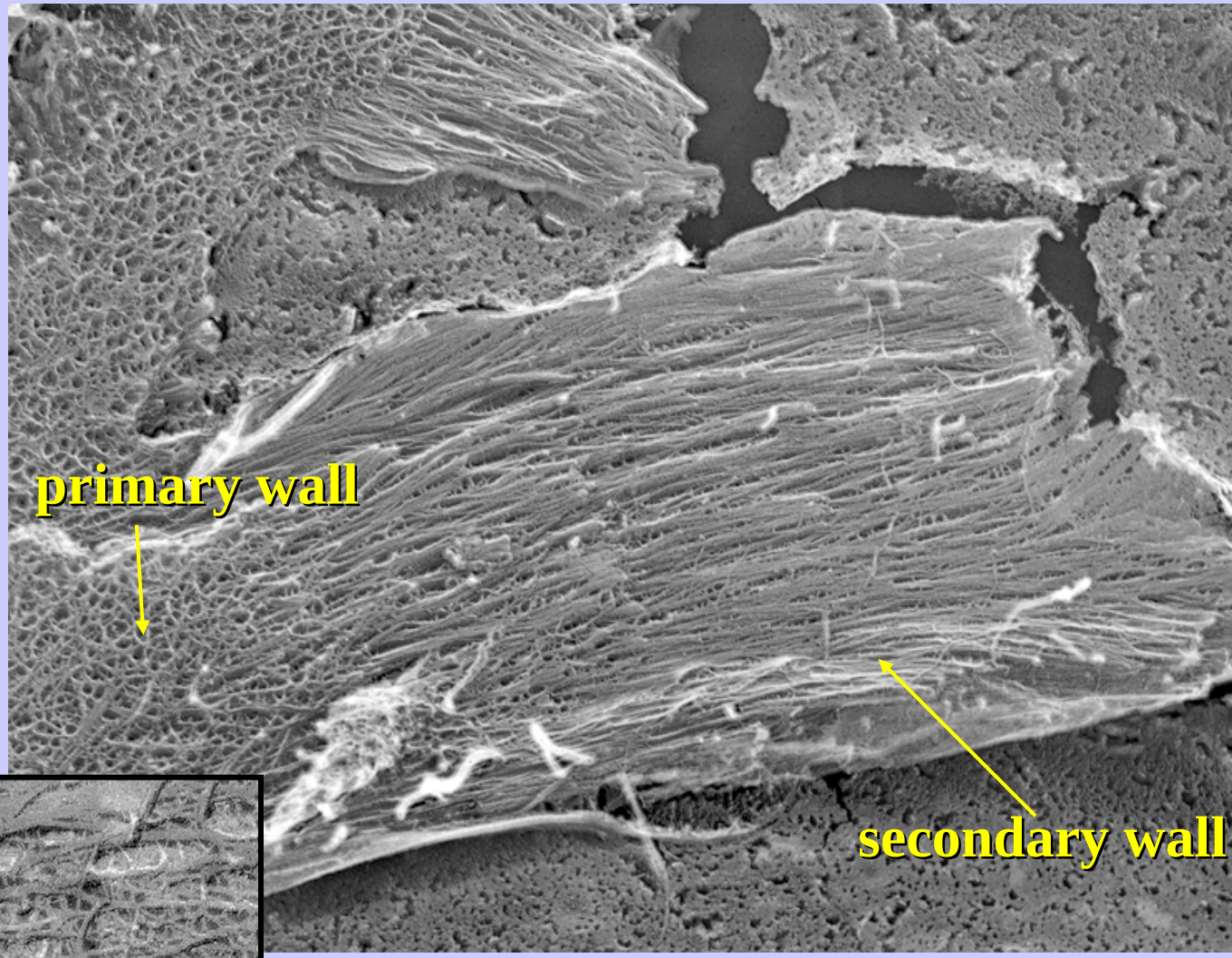
d) Revealing nanoscale details of processed fiber

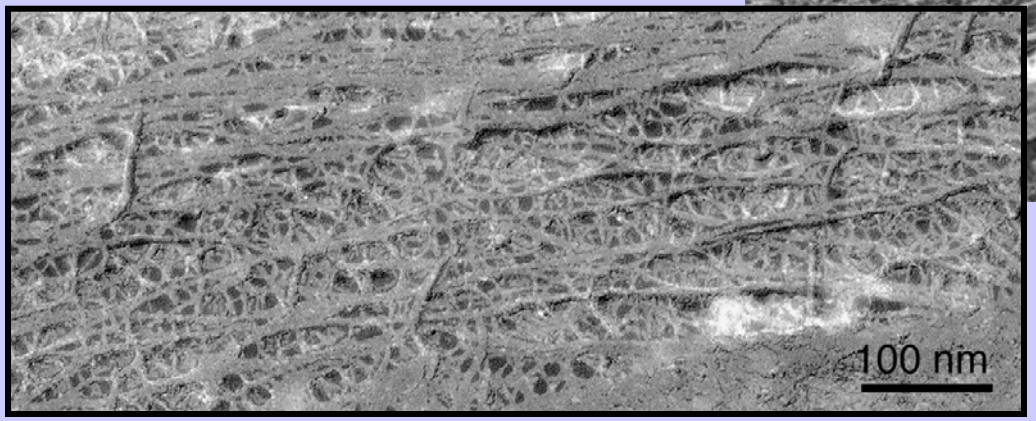
IV. New initiatives in immunolabeling

I. Why does immunolabeling matter in nanotechnology?

The nanoscale structure and properties of fiber cell walls are the basis of the forest products industry.

Immunolabeling can reveal mechanisms of biogenesis and correlate chemistry with structure at the nanoscale.





TEM images of metal-shadowed walls of unlignified wood-like cells prepared by freeze-fracture and deep etching.

Opportunities in nanotechnology that can be facilitated through research involving immunolabeling:

1) **Innovation of new products** that fully exploit the native biostructure requires understanding nanoscale properties of the raw material as they relate to product performance.

2) **Product potential and performance can be optimized** in the context of changes in nanoscale properties that occur during raw material processing and product manufacture.

3) **Beneficial changes in nanoscale properties of raw materials** through genetic engineering are expected to be possible, given more understanding of biogenetic processes at the nanoscale.

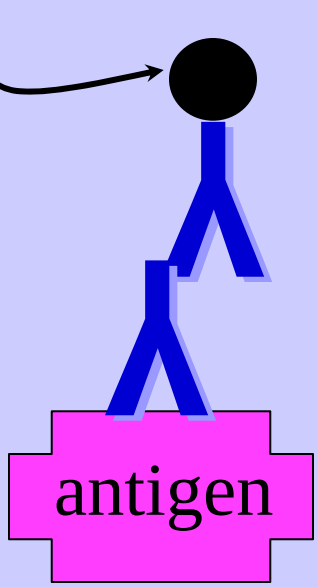
4) **Biomimetic product innovation** can be based on prototypes of native structures once they are understood at the nanoscale.

II. What is immunolabeling?

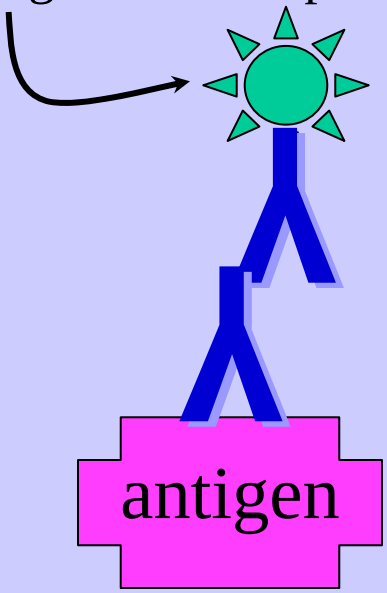
Immunolabeling allows analysis of the distribution of selected chemical epitopes within fiber cell walls, pulp, or reassembled composites.

Immunolabeling uses specific antibodies, which are proteins, to tag an antigen in a complex mixture.

Colloidal gold tag visible in the electron microscope.



Fluorescent tag visible in the light microscope.

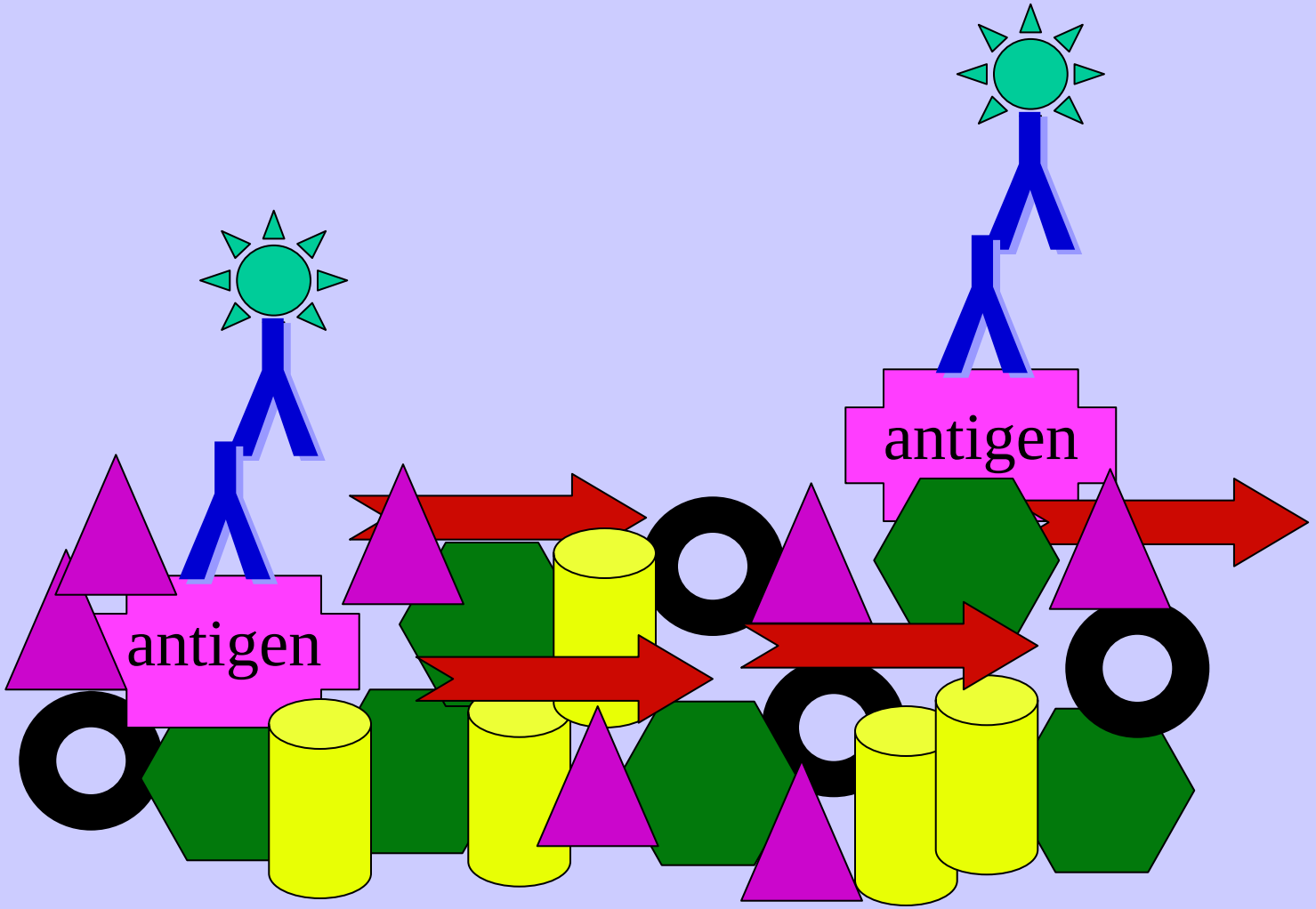


Indirect immunofluorescence uses two specific antibodies:

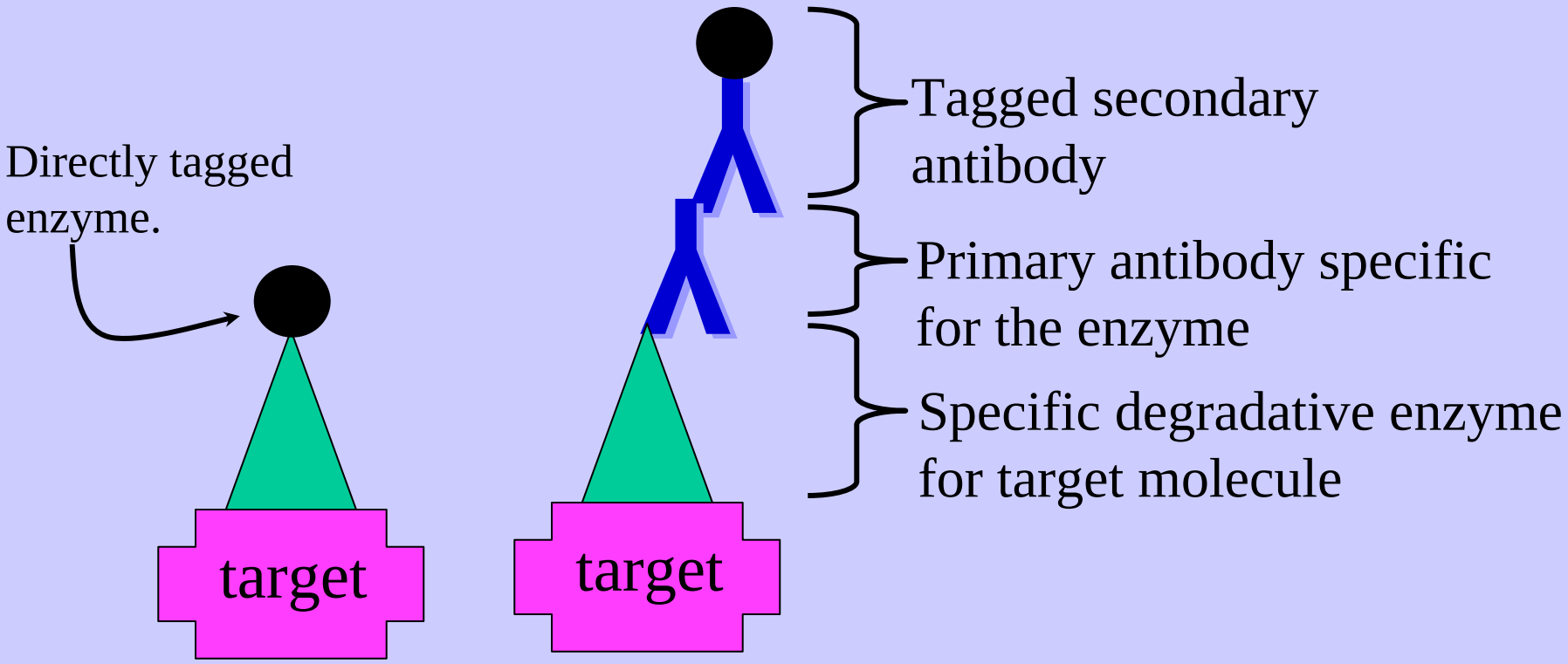
(2) Secondary antibody tagged with a visible marker.

(1) Primary antibody generated by injecting an animal with the antigen.

The detectable marker, in this case green fluorescence , only appears where the antigen is located.



Variations based on use of specific degradative enzymes may be useful for studies of plant cell walls.



The specificity of an antibody is determined by shape recognition between the protein and its antigen, which may be another protein, an oligo- or poly-saccharide, a lignin monomer or fragment, etc.

Therefore, the use of immunolabeling in plant science depends on the generation of specific antibodies to epitopes of interest.

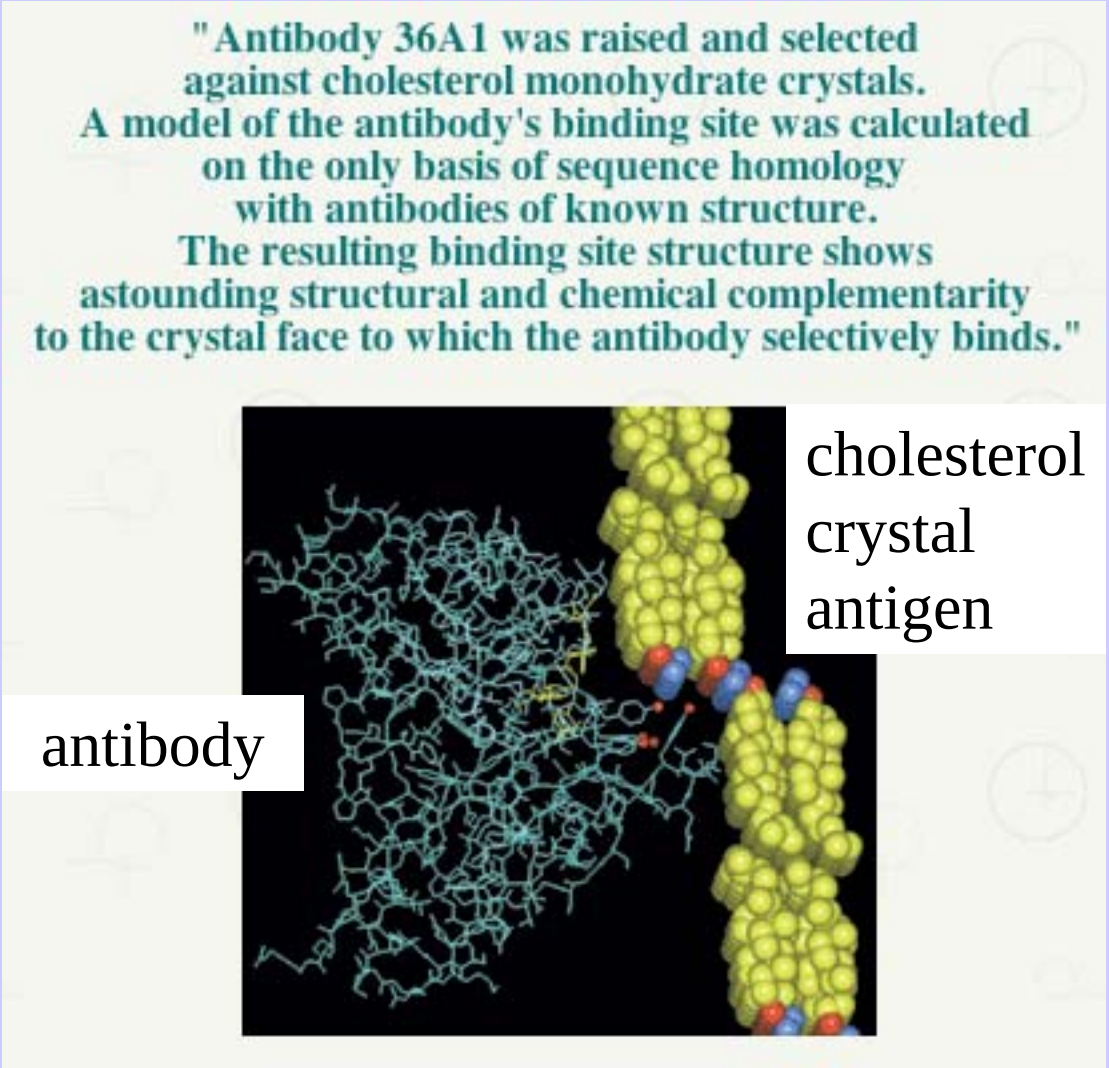
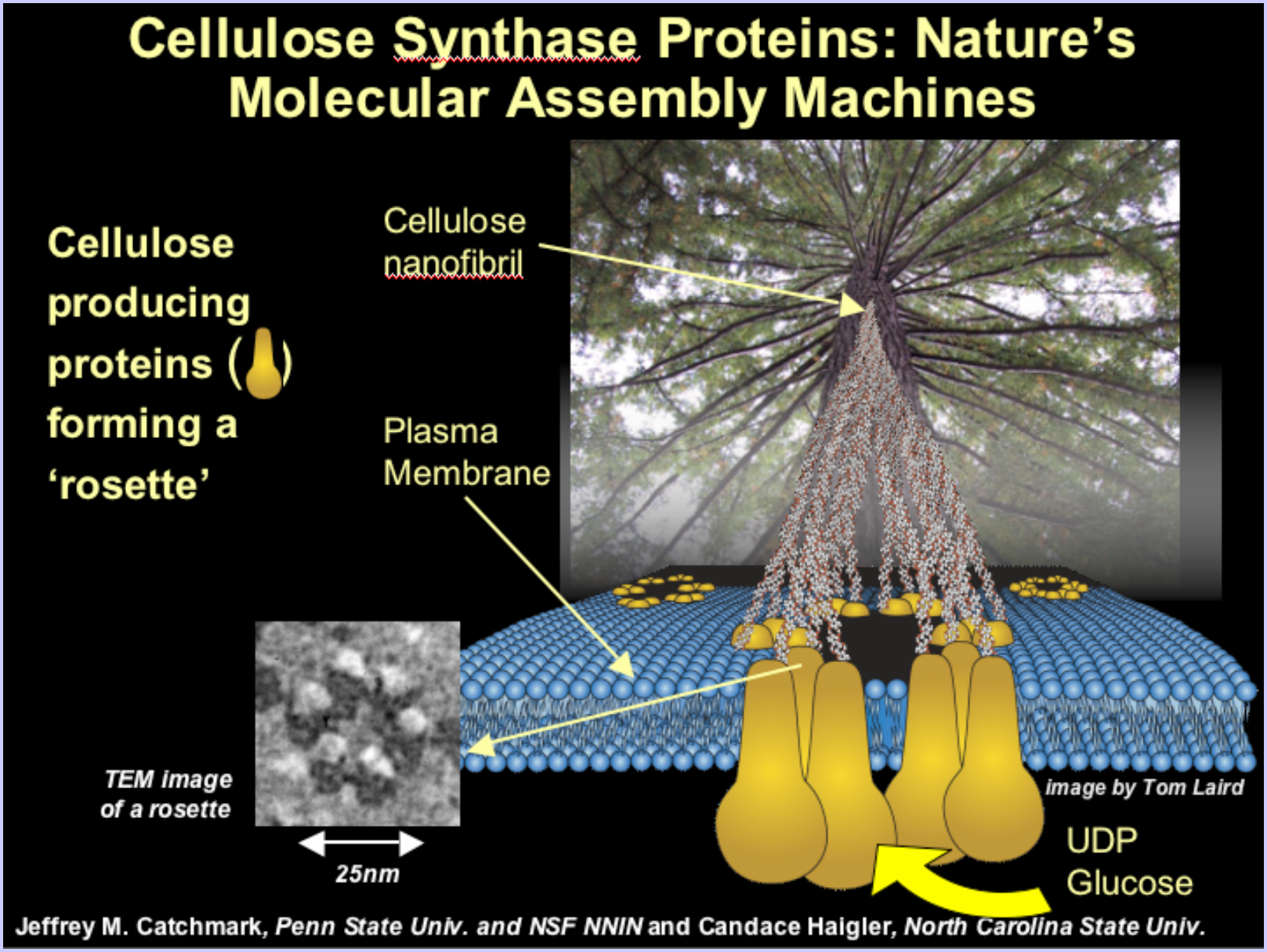


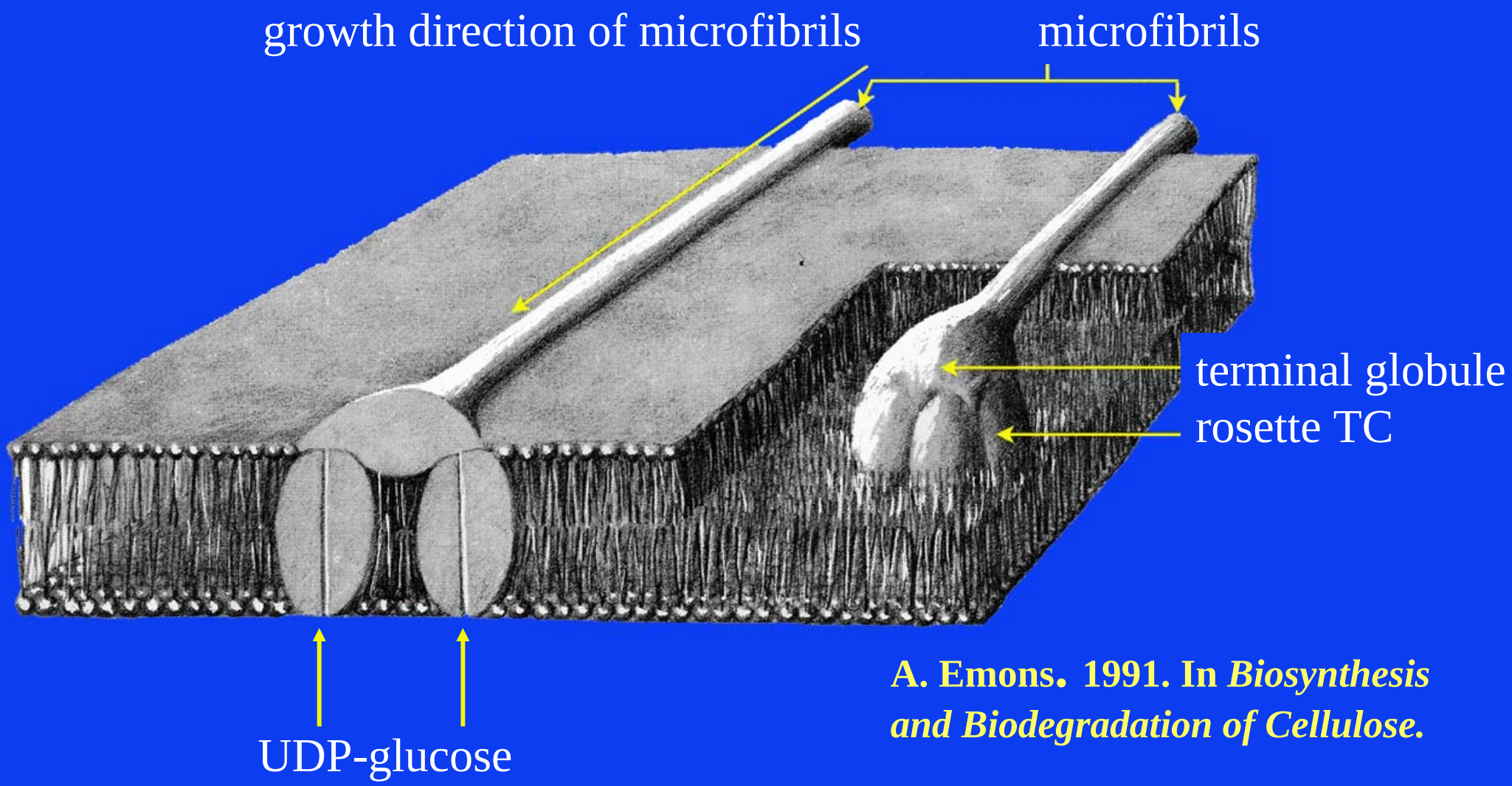
Illustration copied from:
www.weizmann.ac.il/.../Addadi/crystal.html

III. Applications of immunolabeling

a) Revealing mechanisms of cellulose biogenesis

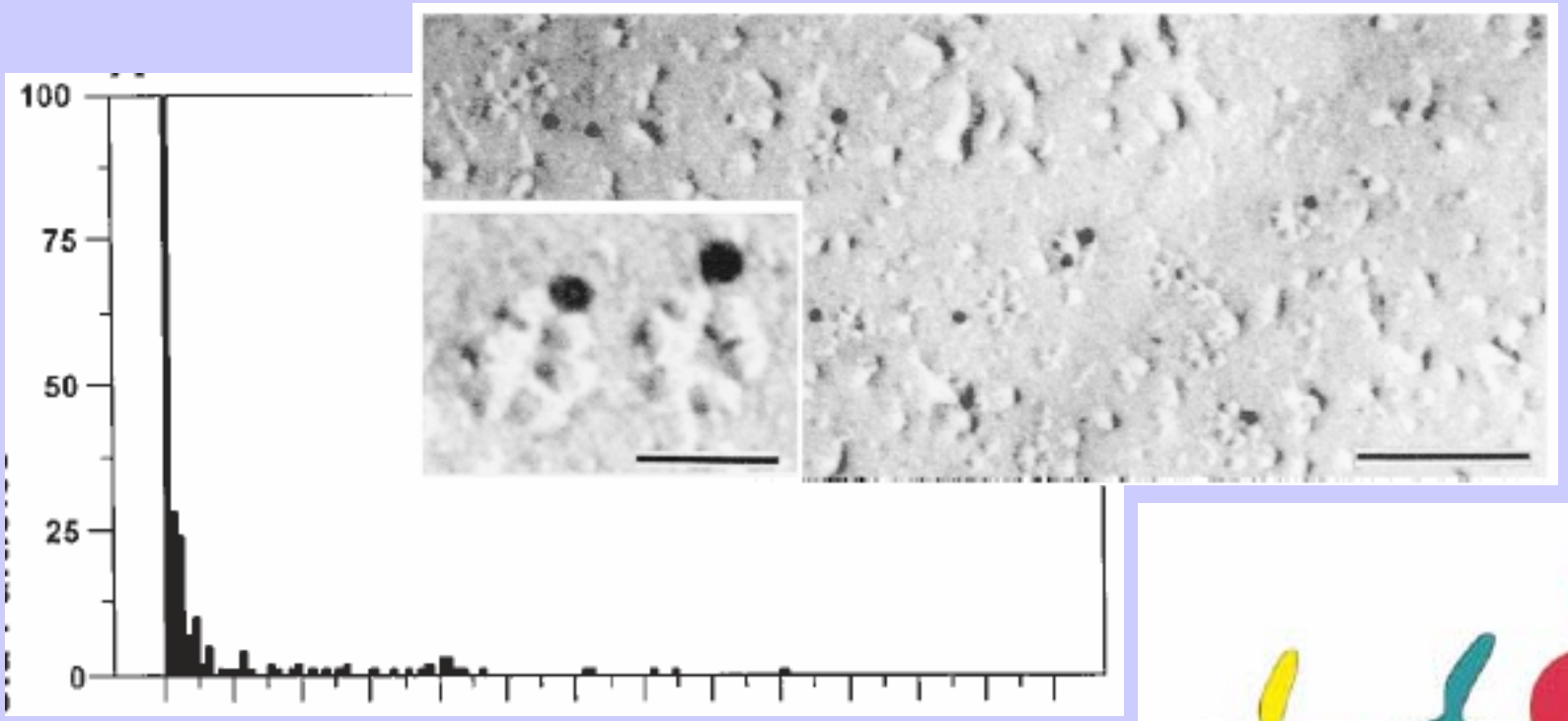


Rosettes are proposed to move within the plasma membrane as they spin out cellulose microfibrils (and there are emerging supportive data).



A. Emons. 1991. In *Biosynthesis and Biodegradation of Cellulose.*

Freeze fracture immunolabeling showed that the rosette contained CesA (cellulose synthase) protein.



Number of colloidal gold particles vs. distance from a rosette (25 nm increments; 217 counted)

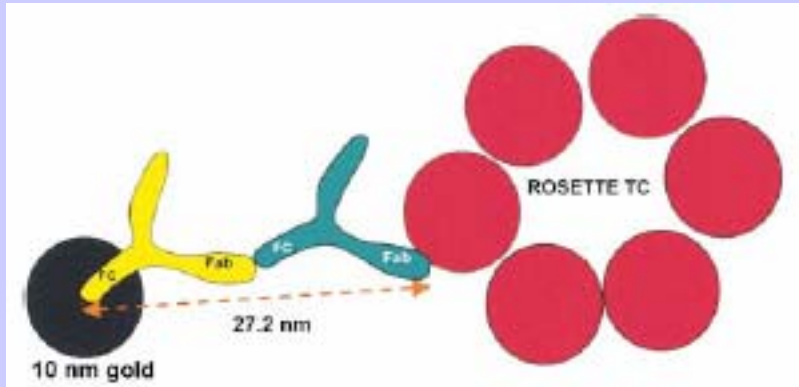
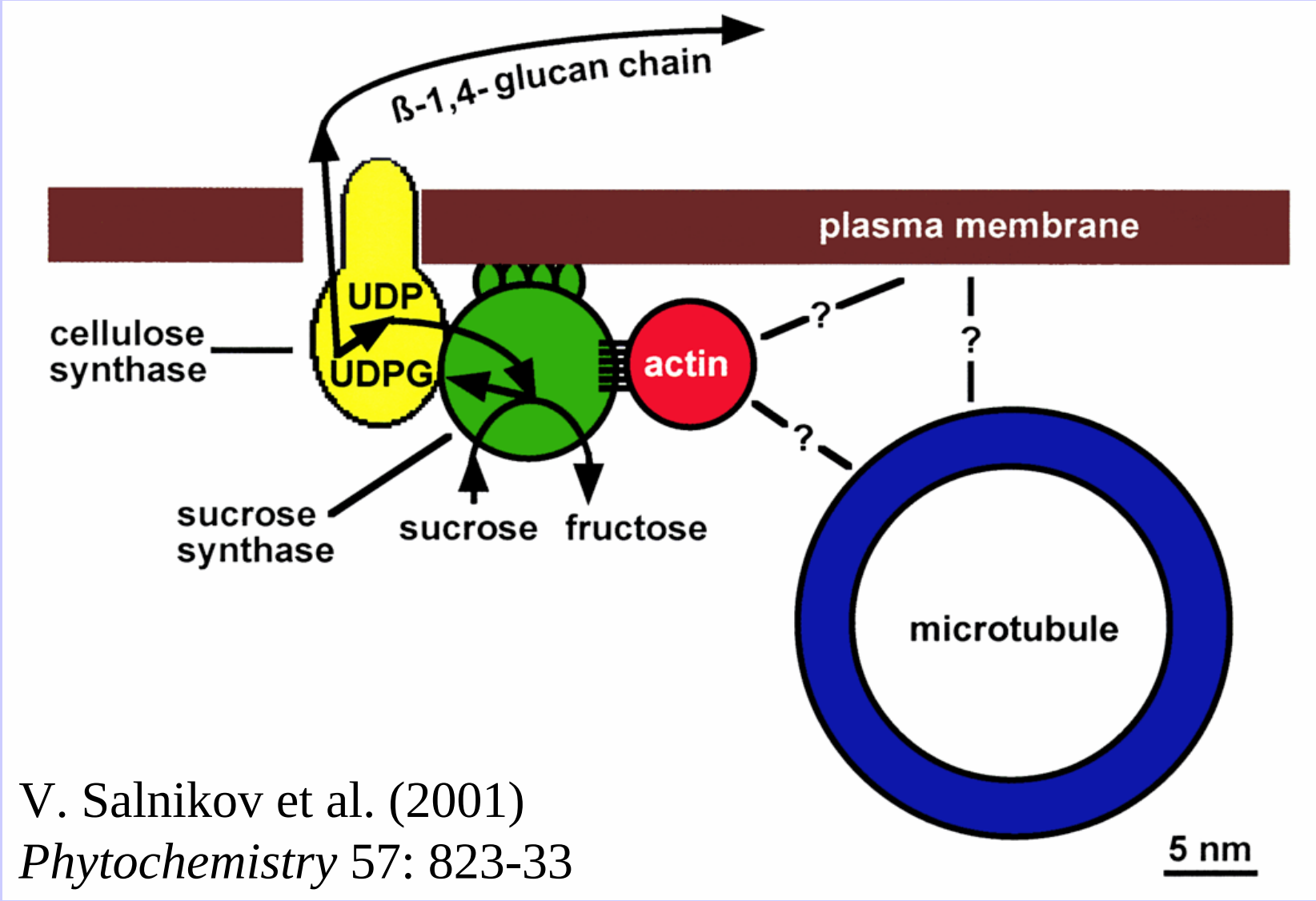


Figure 2. Scale Model Depicting Typical Primary and Secondary Antibody Dimensions Relative to the 10-nm Gold and Rosette TC Dimensions.

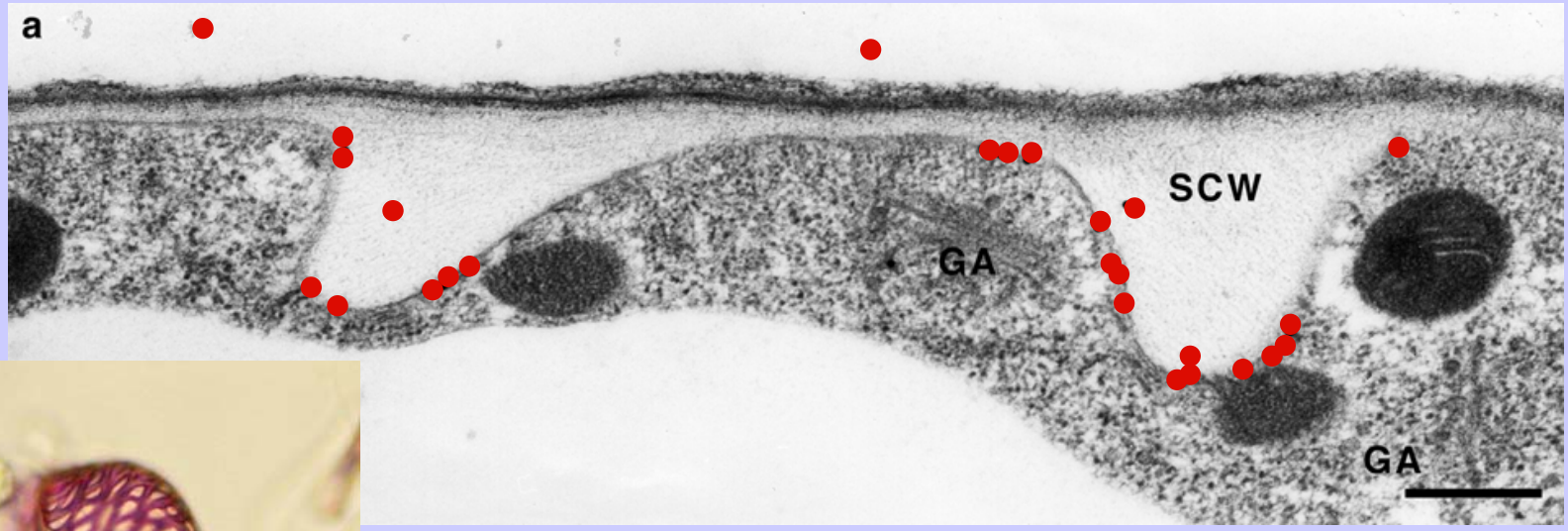
S. Kimura et al. (1999) *The Plant Cell* 11: 2075-2085

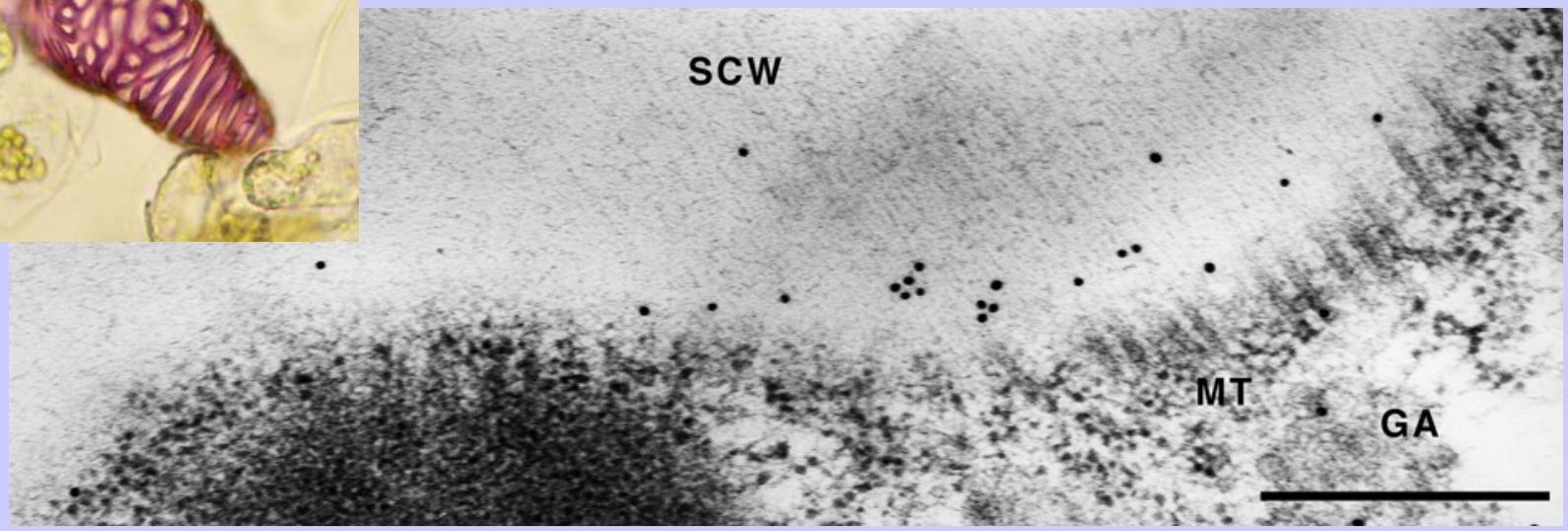
Cellulose synthesis does not depend on only one protein, as was also shown by immunolabeling (in addition to genetic analysis).



V. Salnikov et al. (2001)
Phytochemistry 57: 823-33

Sucrose synthase was specifically located at patterned sites of secondary wall cellulose synthesis in tracheary elements.

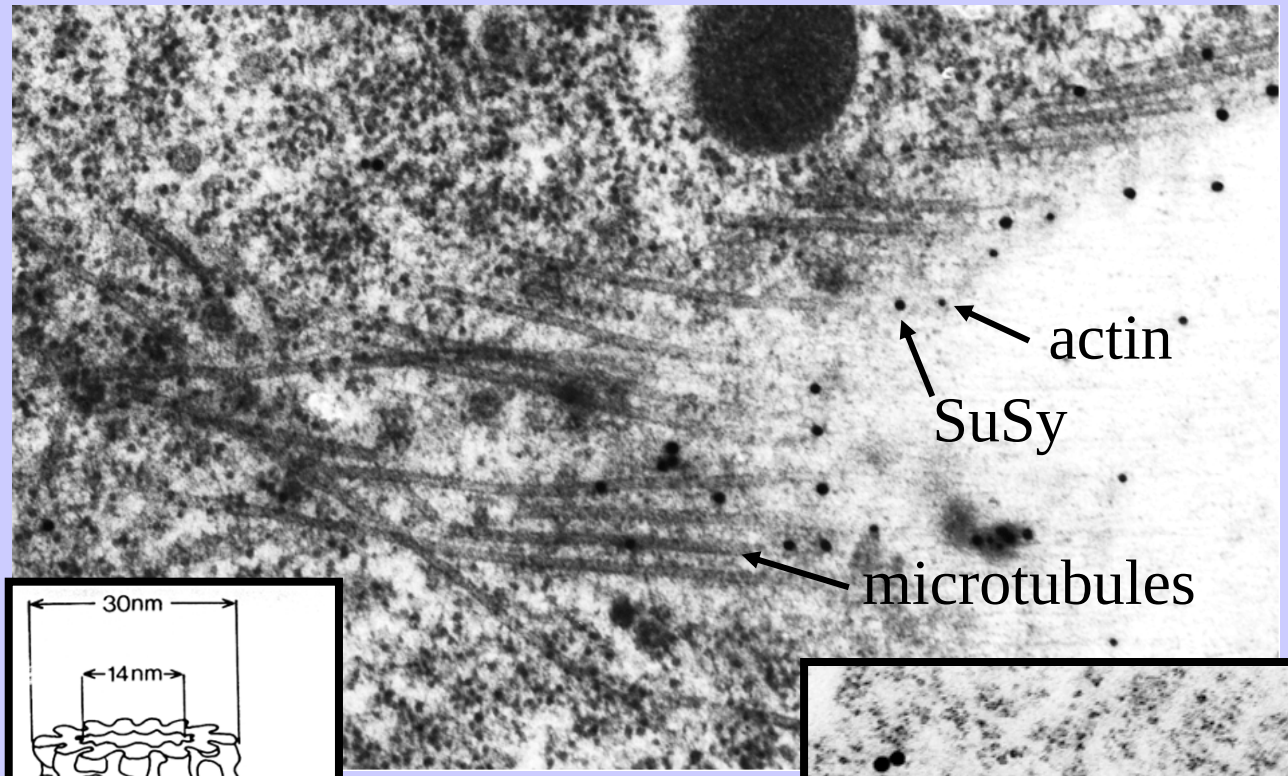




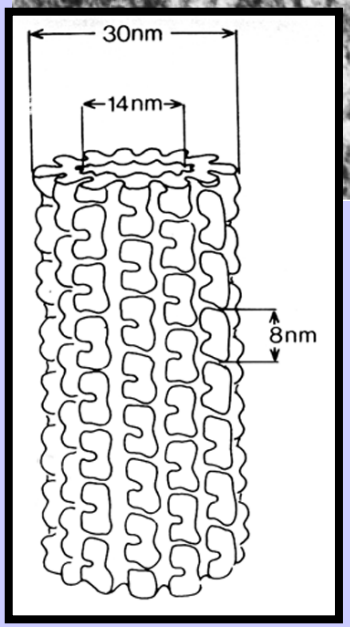
V. Salnikov et al.

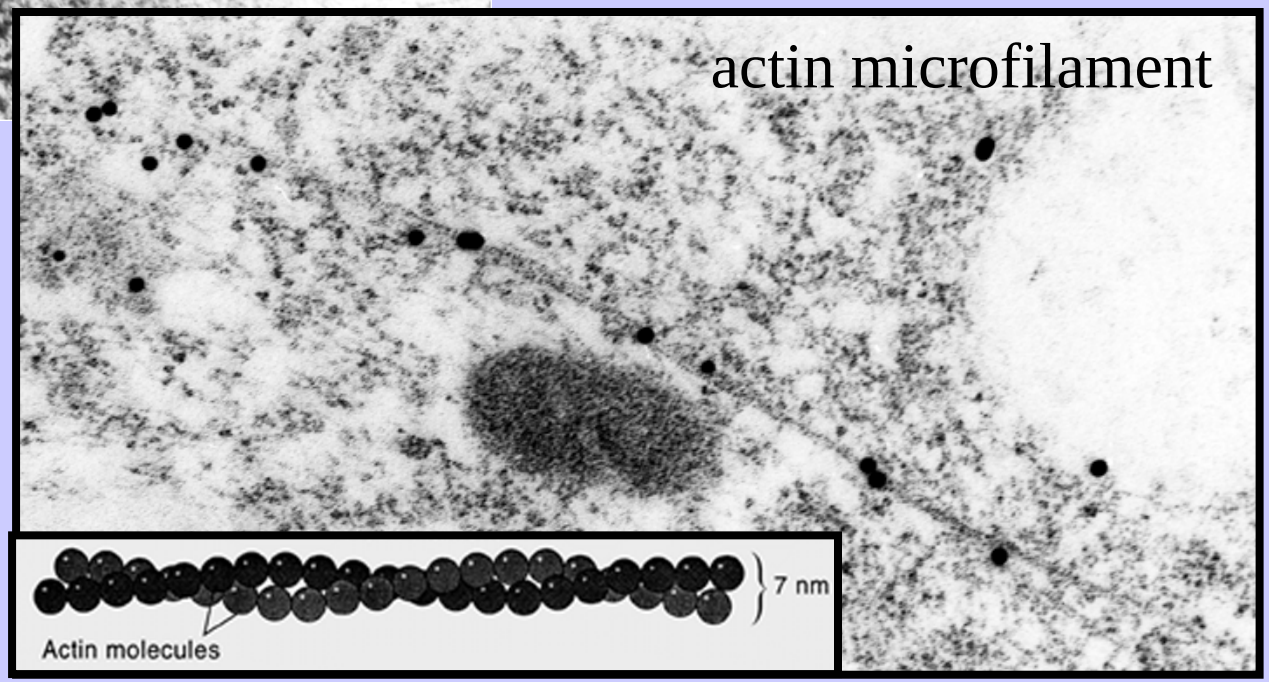
Two secondary wall thickenings (SCW) in cross-section. SuSy is indicated by particles of colloidal gold.

Tangential sections showed that SuSy existed above the cortical micro-tubules (MTs) in the vicinity of the plasma membrane.



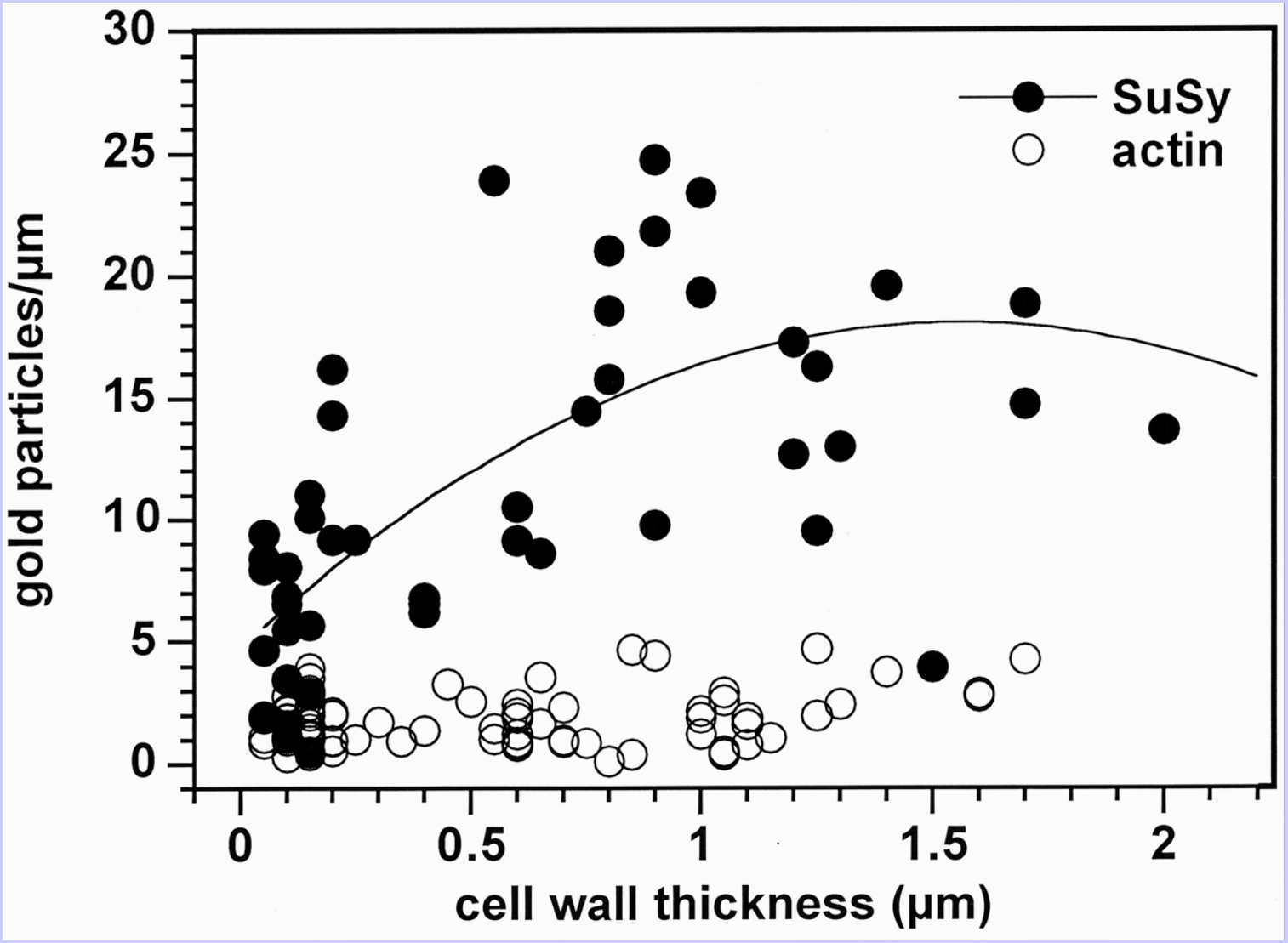
Actin was also located near SuSy and the microtubules, as indicated by double labeling with two sizes of colloidal gold.





V. Salnikov et al.

Distribution of sucrose synthase and actin relative to regions of primary and secondary wall in tracheary elements

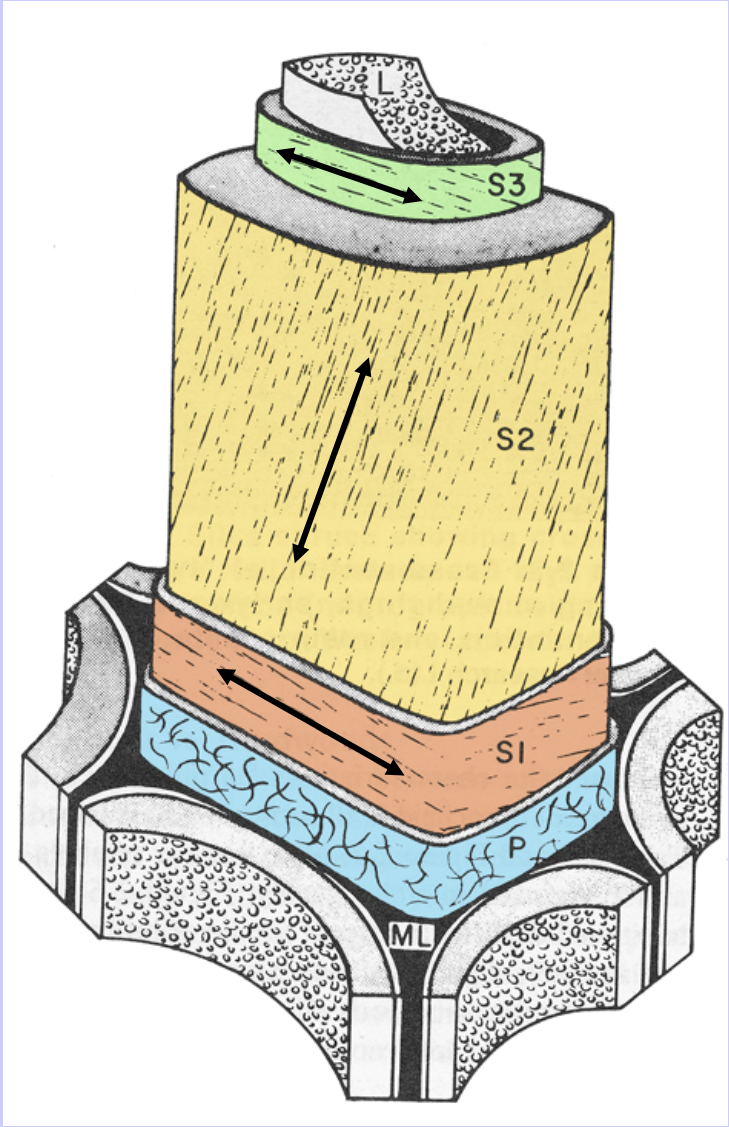


Actin may be part of a complex including SuSy and microtubules, but actin alone cannot explain the patterned distribution of SuSy.

V. Salnikov et al.

III. Applications of immunolabeling

b) Illustrating spatial chemistry of the native raw material



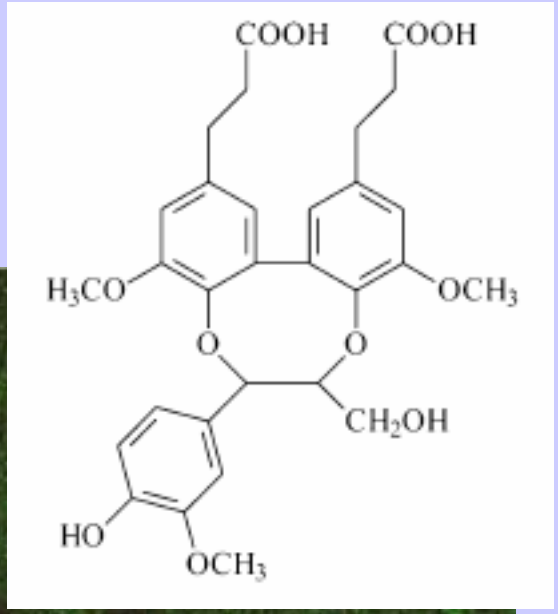
Wood fiber walls are natural composites of cellulose, non-cellulosic polysaccharides, protein, and lignin.

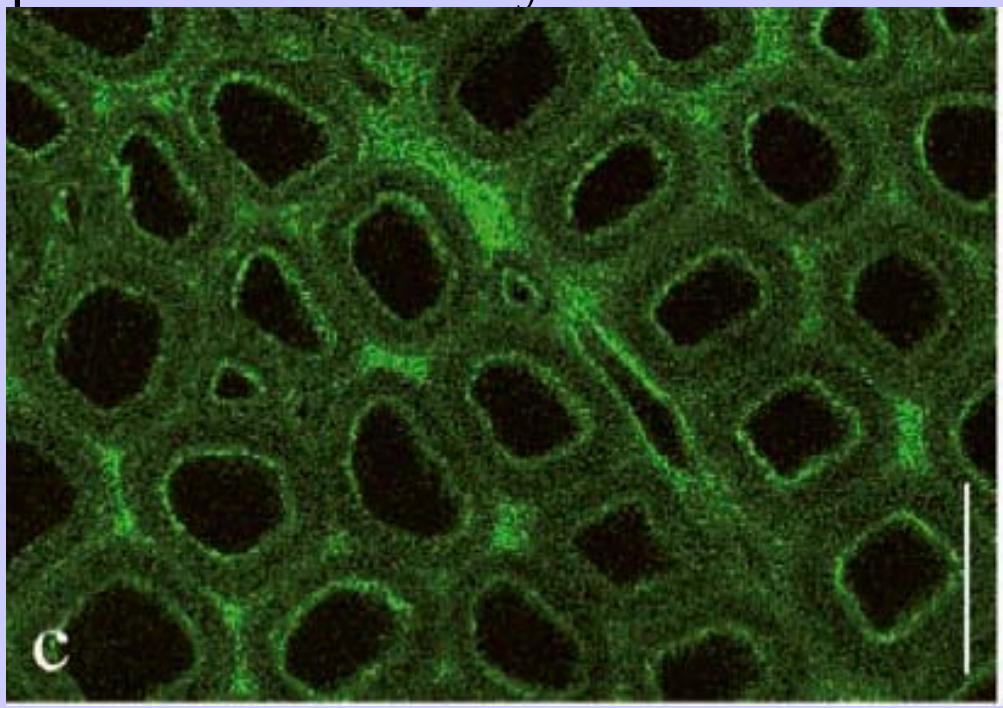
The native structure affects product performance, so it is necessary to understand it as fully as possible, including at the nanoscale.

Diagram adapted from G.A. Smook (1992) *Handbook for Pulp and Paper Technologists.*

Fluorescence detection methods provide an overview of which cells contain an epitope.

The condensed lignin substructure, dibenzodioxocin 5-5-O-4 , localized in spruce tracheids, mainly the inner part of the secondary wall and middle lamellae.



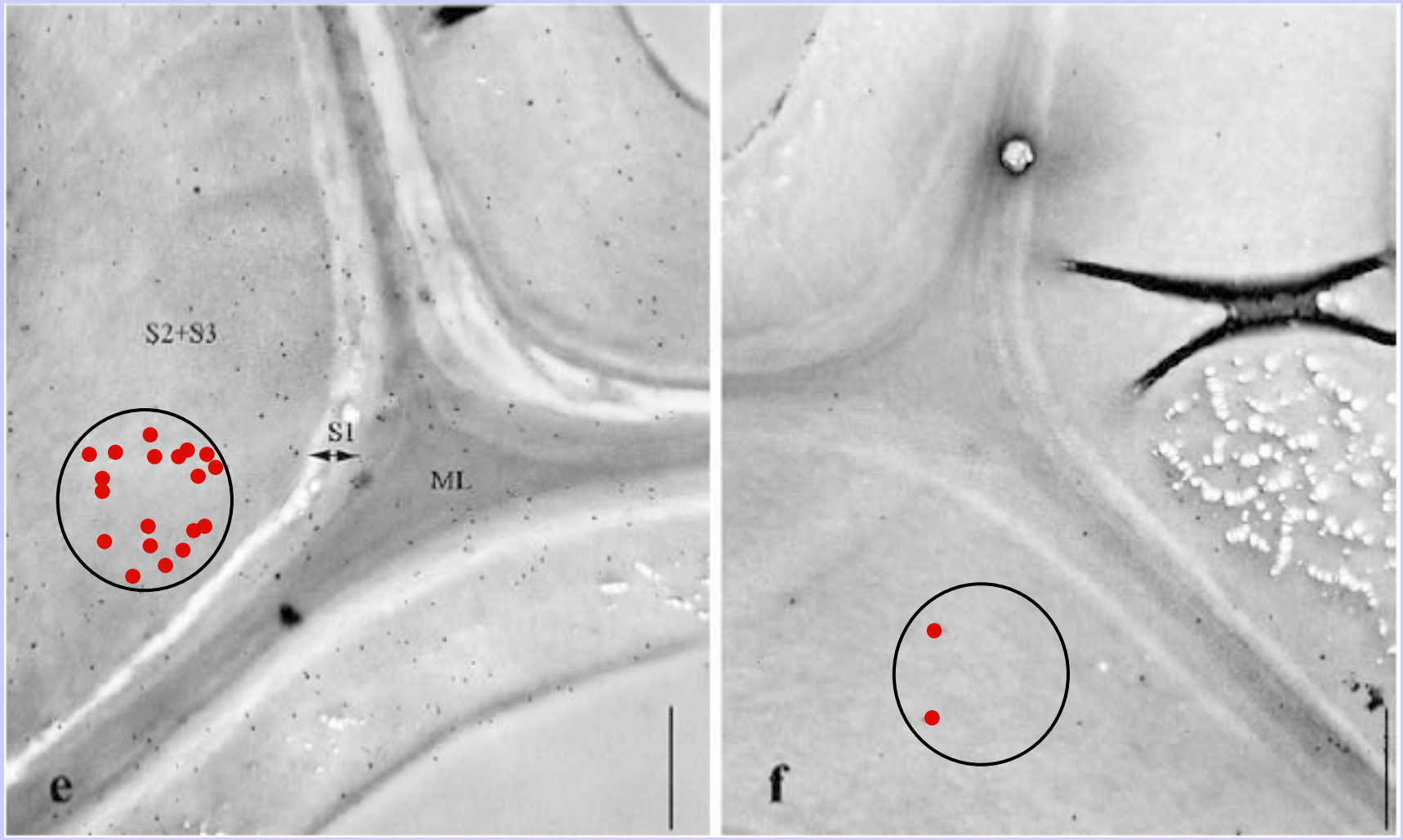


Positive signal from antiserum.

Background fluorescence in pre-immune serum control.

E.M. Kukkola (2004) *Planta* 218: 497-500

TEM immunolabeling showed that the epitope was also found in the S2 and S3 layers.



Positive signal from antiserum.

Background labeling in pre-immune serum control.

E.M. Kukkola (2003) *Planta* 217: 229-237

Specific antibodies to several different lignin epitopes have been available for many years.

K. Ruel, In: (2004) *COST Action E20 Wood Fibre Cell Wall Structure*, p. 131-140

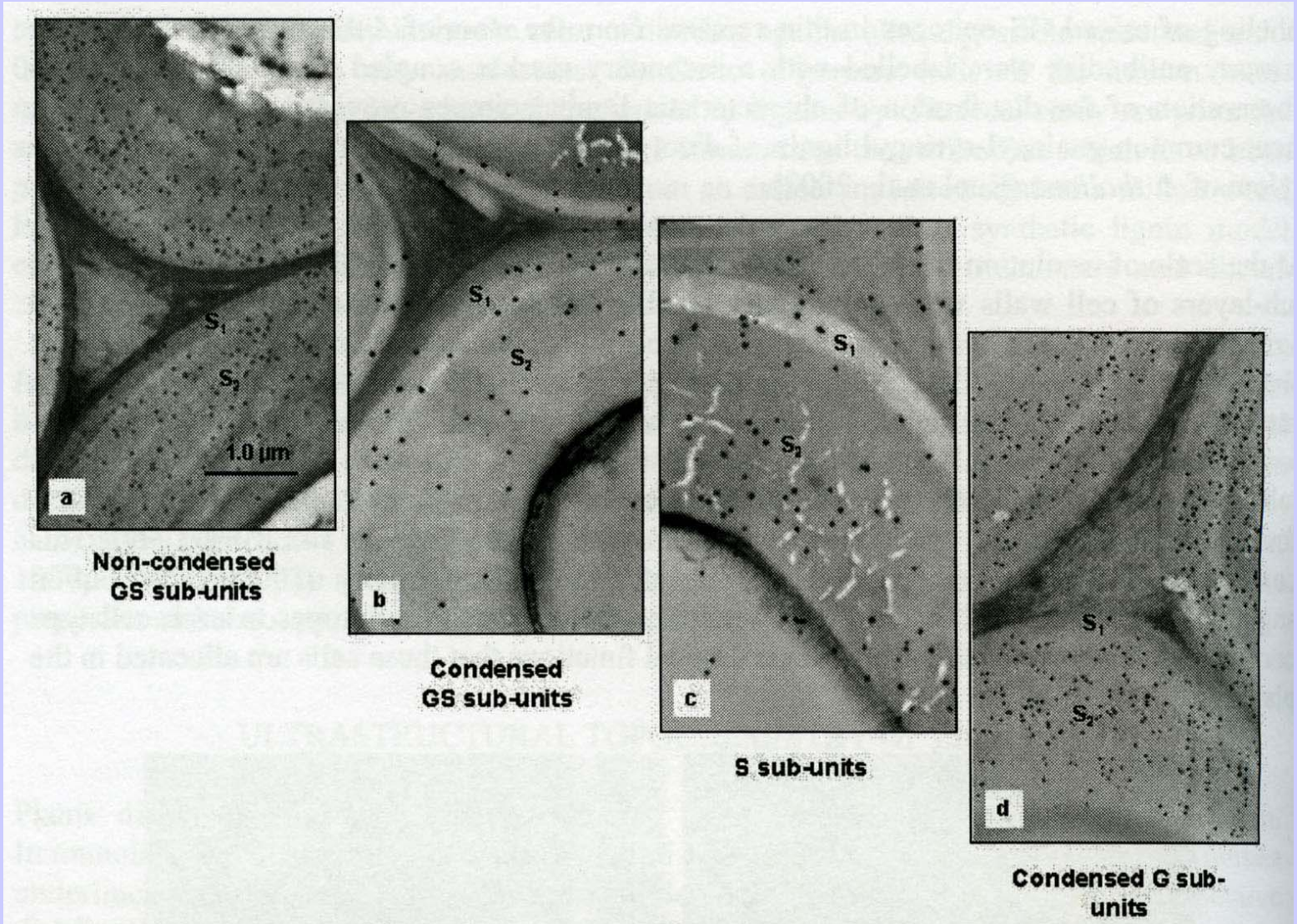


Fig. 3 a-d. Immuno-labelling of fibre walls from the wood of *Populus tremula* with four different lignin antibodies. -a : non-condensed GS epitopes. -b :condensed GS epitopes. -c : Syringyl (S) epitopes. -d :condensed homoguaiacyl (G) epitopes .

Immunolabeling is useful in analysis of transgenic plants with altered lignin content.

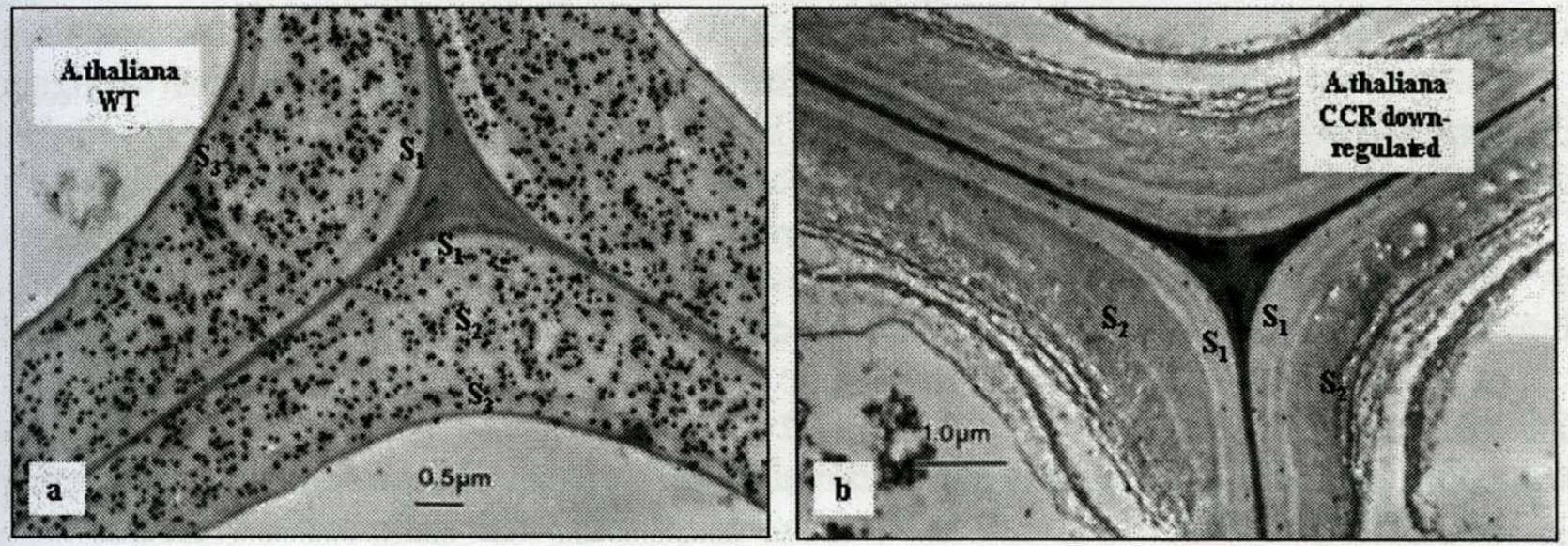
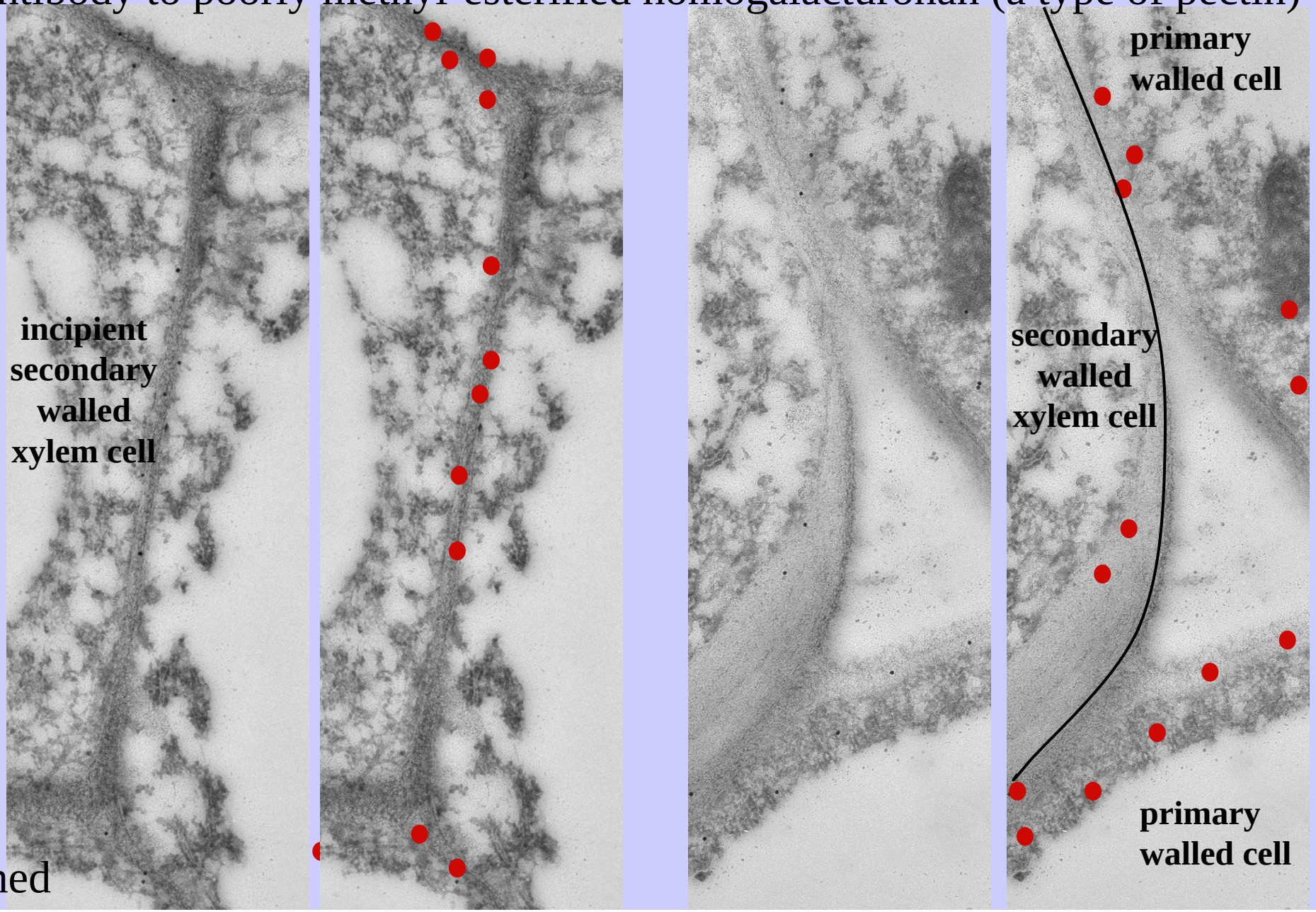


Fig. 4 a-b. Consequence of down-regulation of cinnamoyl-CoA-reductase (CCR) activity in *A. thaliana*. -a: normal plant: compactness of S_1 and S_2 sub-layers and strong labelling for non-condensed mixed GS lignin sub-units -b: fibres from the ASCCR plant exhibiting a loss of cohesion in the inner part of S_2 and a very poor labelling restricted to S_1.

K. Ruel, In: (2004) *COST Action E20 Wood Fibre Cell Wall Structure*, p. 131-140

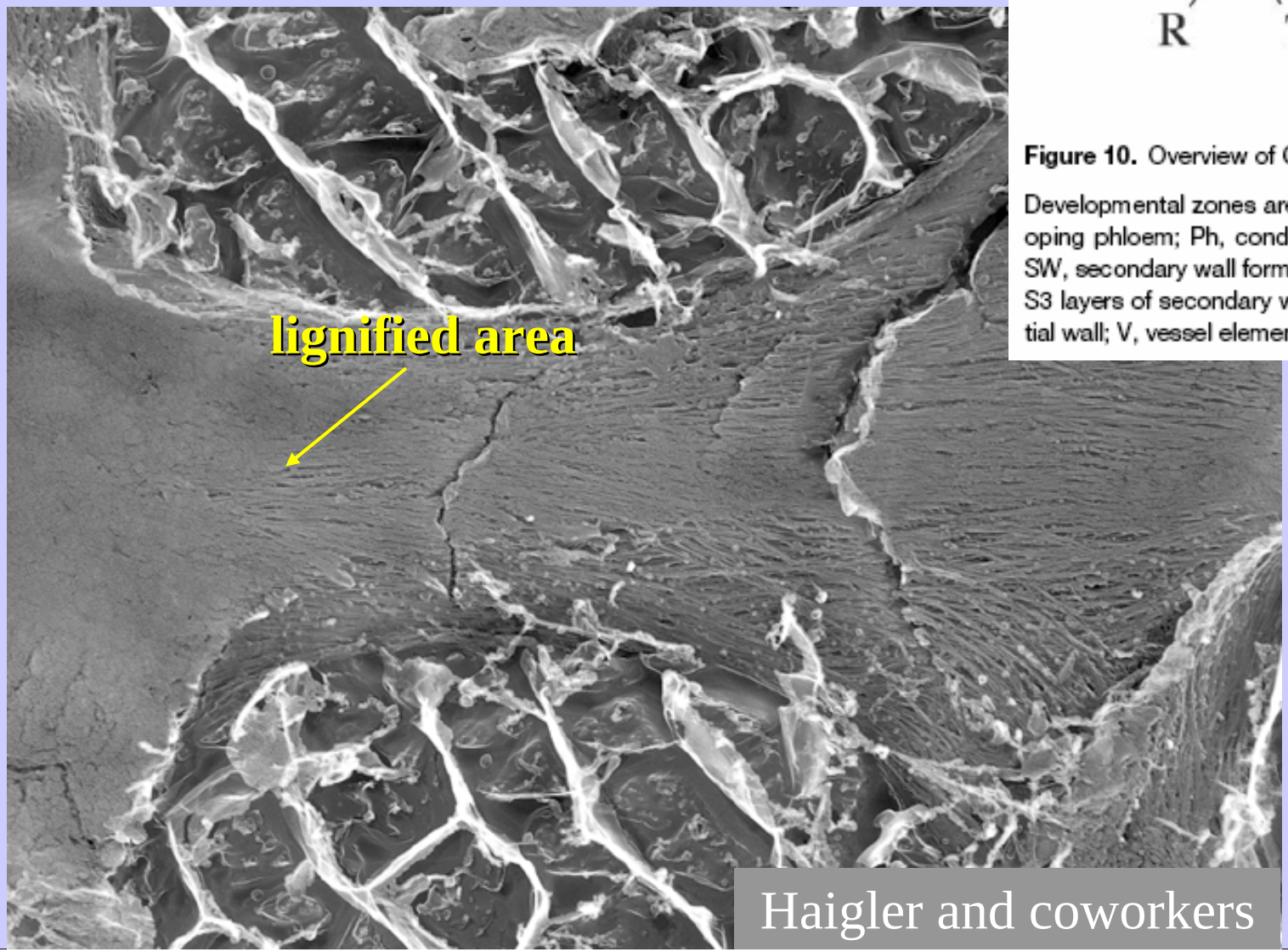
Immunolabeling of polysaccharide components:

JIM5 antibody to poorly methyl-esterified homogalacturonan (a type of pectin)



Avci and Haigler, unpublished

Immunolabeling of polysaccharide and protein epitopes of native fiber must be done with developing tissue before lignin obscures other molecules.



Haigler and coworkers

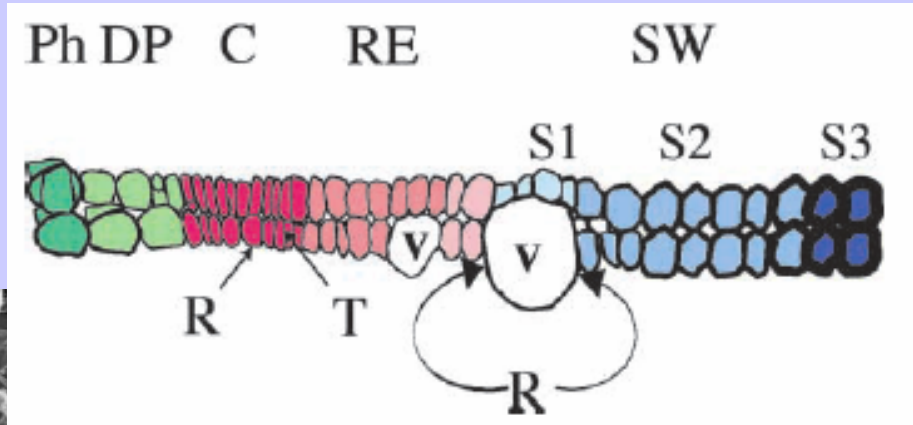


Figure 10. Overview of Cambial Region Tissues.

Developmental zones are color coded: C, cambial zone; DP, developing phloem; Ph, conducting phloem; RE, radial expansion zone; SW, secondary wall formation zone in which successive S1, S2, and S3 layers of secondary wall are deposited. R, radial wall; T, tangential wall; V, vessel element.

Bourquin et al. 2002

Fiber is formed in a predictable developmental sequence, which aids analysis of unlignified cells.

Immunolabeling of xyloglucan in developing fiber

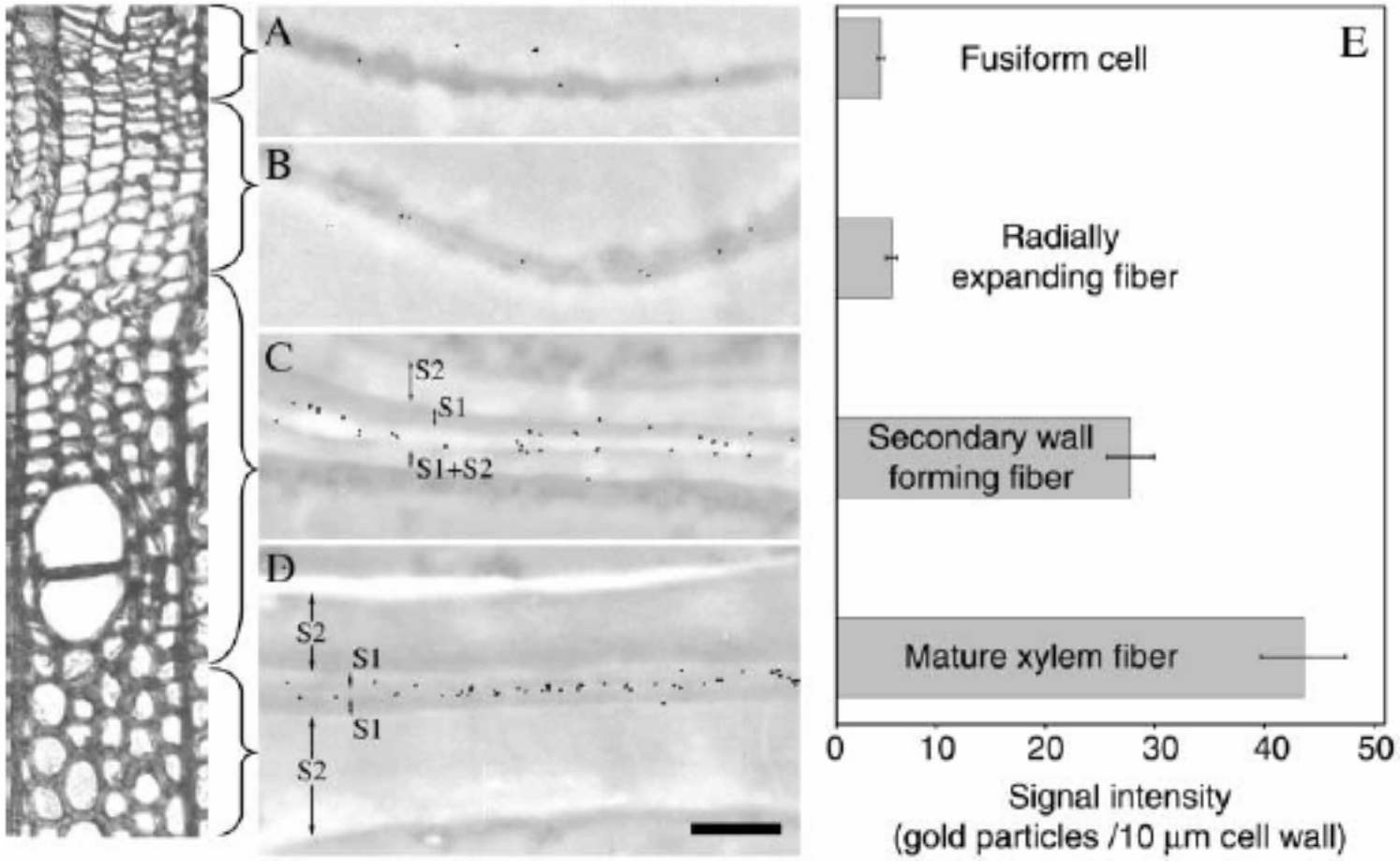


Figure 3. Transmission Electron Microscopy Immunolocalization of XG in the Walls of Developing Xylem Fibers Using the Monoclonal Antibody CCRC-M1.

(A) to **(D)** Tangential walls of fibers in the meristematic stage **(A)**, the radial expansion stage **(B)**, the early secondary wall deposition stage **(C)**, and the almost mature stage **(D)**. Note the accumulation of the label at the primary/S1 layer boundary. Bar in **(D)** = 2 µm for **(A)** to **(D)**.
(E) Quantification of the label in tangential walls of developing fibers. Scores were taken from 10 random locations of tangential walls in 10 different cells for each stage. Bars indicate standard errors.

V. Bourquin et al. (2002) *Plant Cell* 14: 3073-88

III. Applications of immunolabeling

c) Correlating fiber wall chemistry with nanostructure

Immunolabeling coupled with freeze fracture/deep etch TEM showed that xyloglucan was preferentially associated with fasciated microfibrils in tobacco cells.

T. Itoh (2002) in *Wood Formation in Trees*, p. 83-98

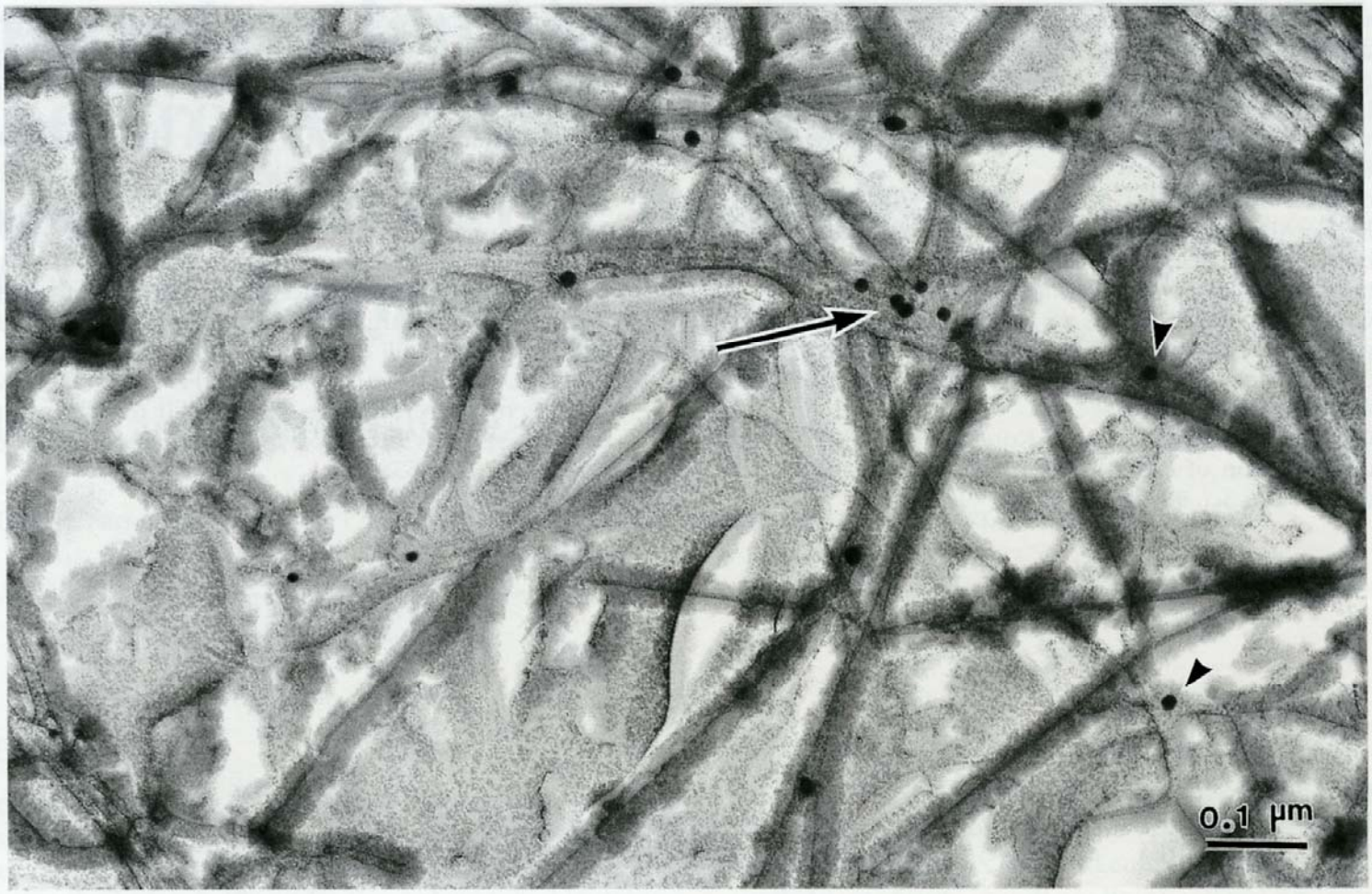
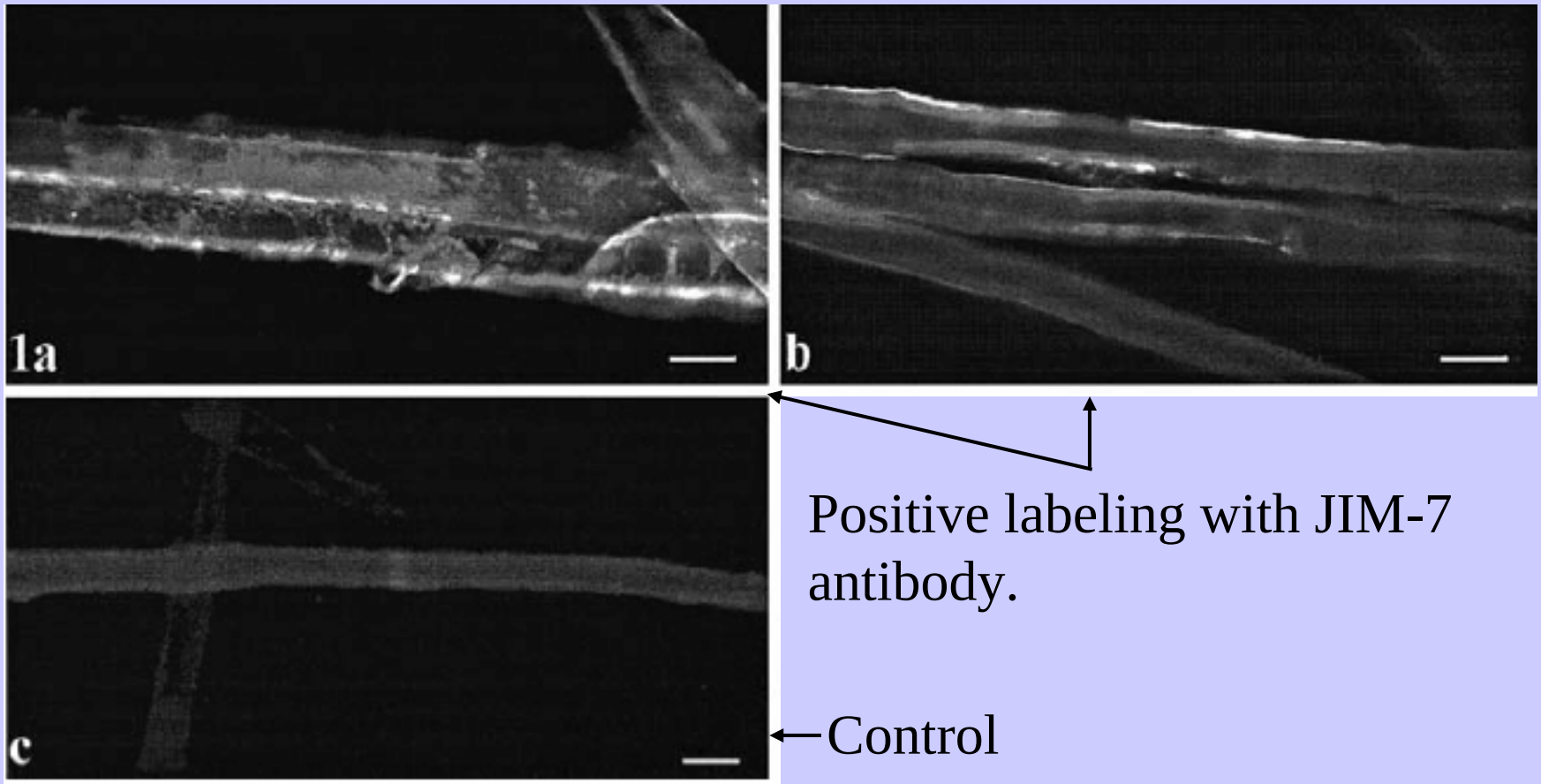


Figure 10 Deep-etched image of 120-hour-old cells of *Nicotiana tabaccum* labelled by the anti-xyloglucan antibodies. The number of gold particles is much higher in 120-hour-old cells than in 3-hour-old cells. Gold particles largely appear on the associated cellulose microfibrils (arrow) and at intersections between crossed microfibrils (arrowheads).

III. Applications of immunolabeling

d) Revealing nanoscale details of processed fiber

In immunofluorescence, chemithermomechanical pulps showed residual pectin that was not found in Kraft pulp (not shown), and this may affect comparative pulp properties.

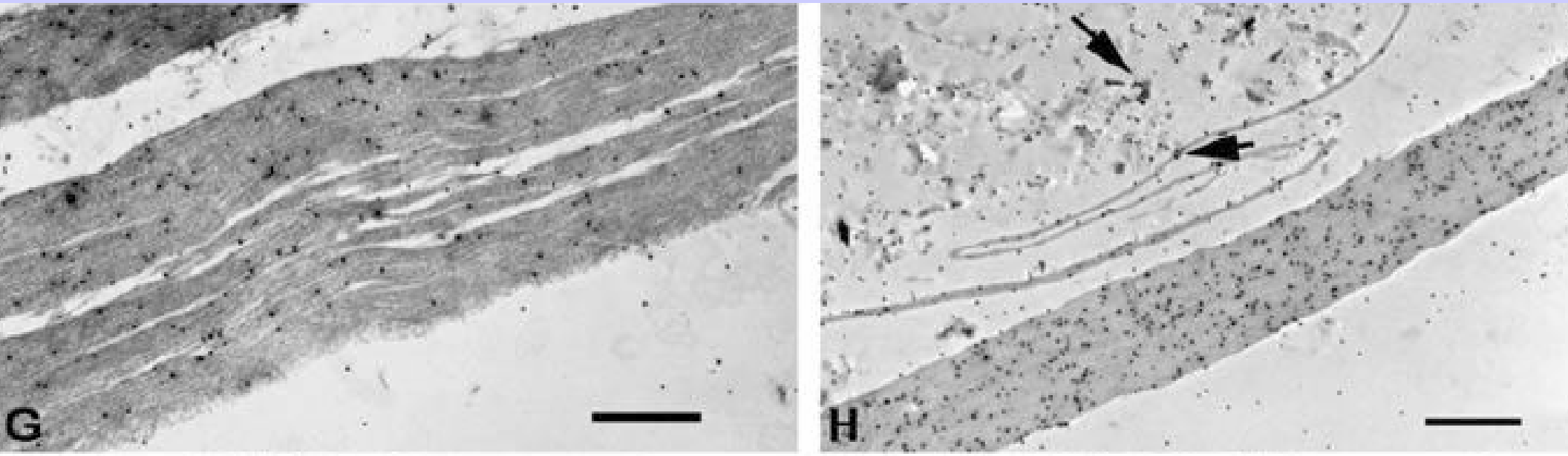


Positive labeling with JIM-7 antibody.

←Control

J. Hafren and G. Daniel (2003) *J. Wood Sci.* 49: 361-365

Immunolabeling of linear xylan within fibers and fines of recycled, refined, pulp



J. Brandstrom et al. (2005) *Holzforschung* 59: 675-680

In replicas, Glucomannan distribution in relation to nanofibrils of Kraft pulp was monitored by the presence of 9 ± 1. 7 nm globules (complex of antibodies and colloidal gold).

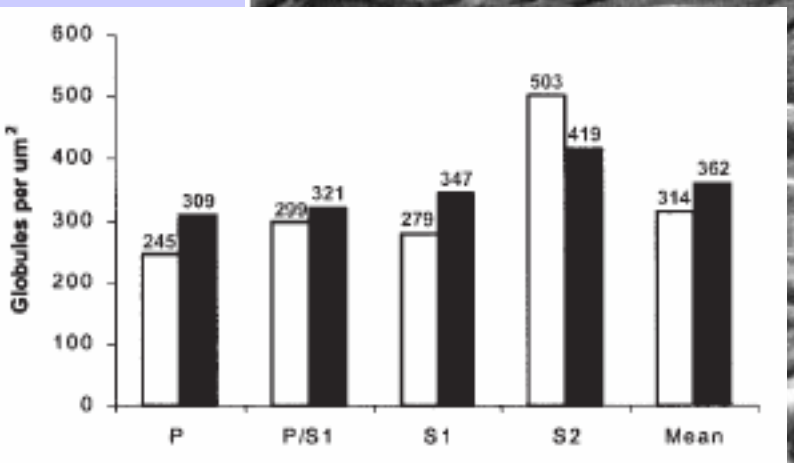
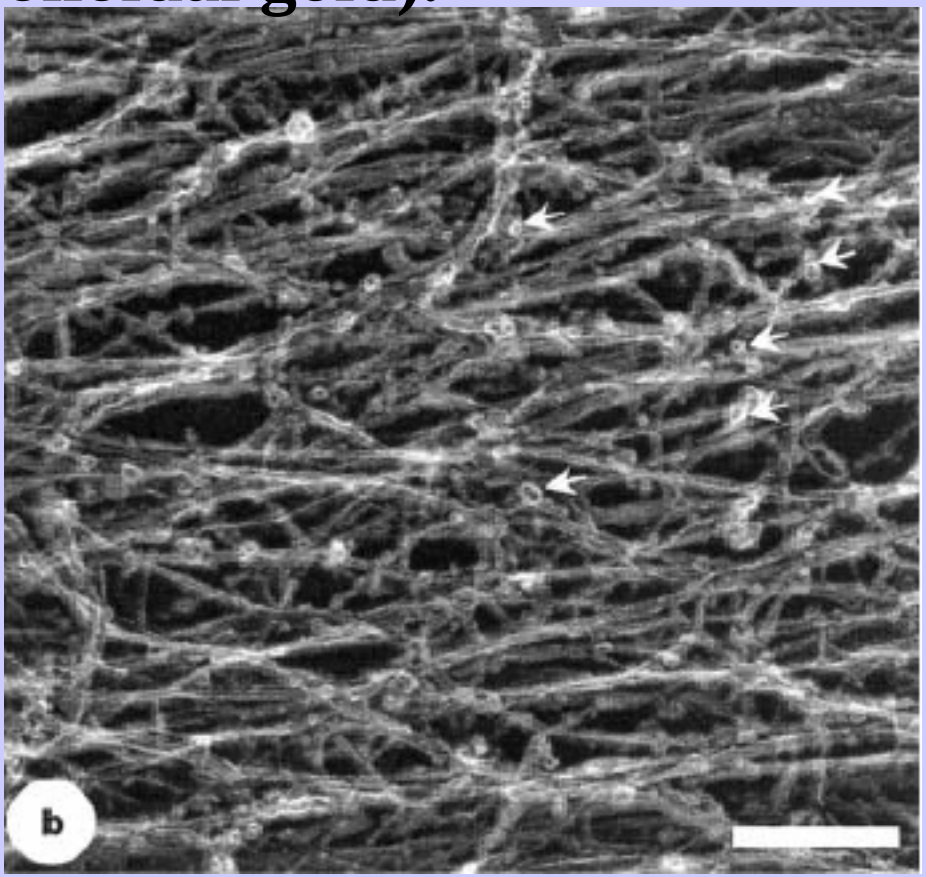


Fig. 5. Frequency of globules per μm² recorded in the different morphological parts of spruce kraft pulp fibres. The globules indicate the distribution of glucomannan on the surface of cellulose macrofibrils for the high (PS, in black) and low (PH, in white) hemicellulose containing pulps. The frequencies of globular structures were not statistically different between the two pulps (p=0.33).

I. Duchesne et al. (2003)
Holzforschung 57: 62-68

Control S1



S1 labeled with antibody to glucomannan, followed by rapid-freeze deep etching and cleaning of the replica with cellulase.

IV. New initiatives in immunolabeling

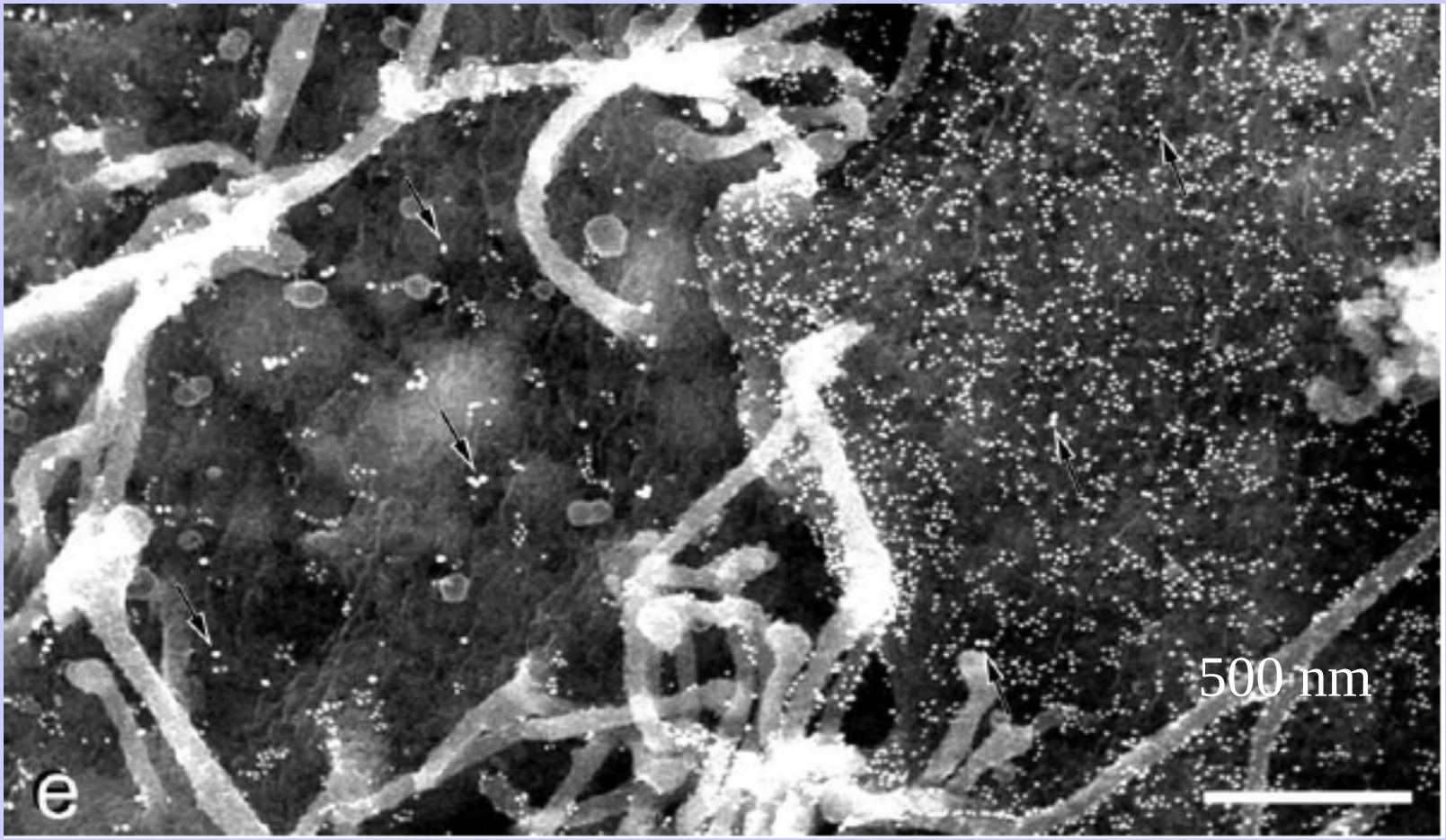
First, a sobering observation…

Almost all the work cited in this presentation using immunolabeling as a tool to understand fiber and pulp nanostructure comes from researchers in Europe or Japan.

What advantages will be gained for other national forest products industries compared to the U.S.A. if this situation continues?

Now, on to new possibilities….

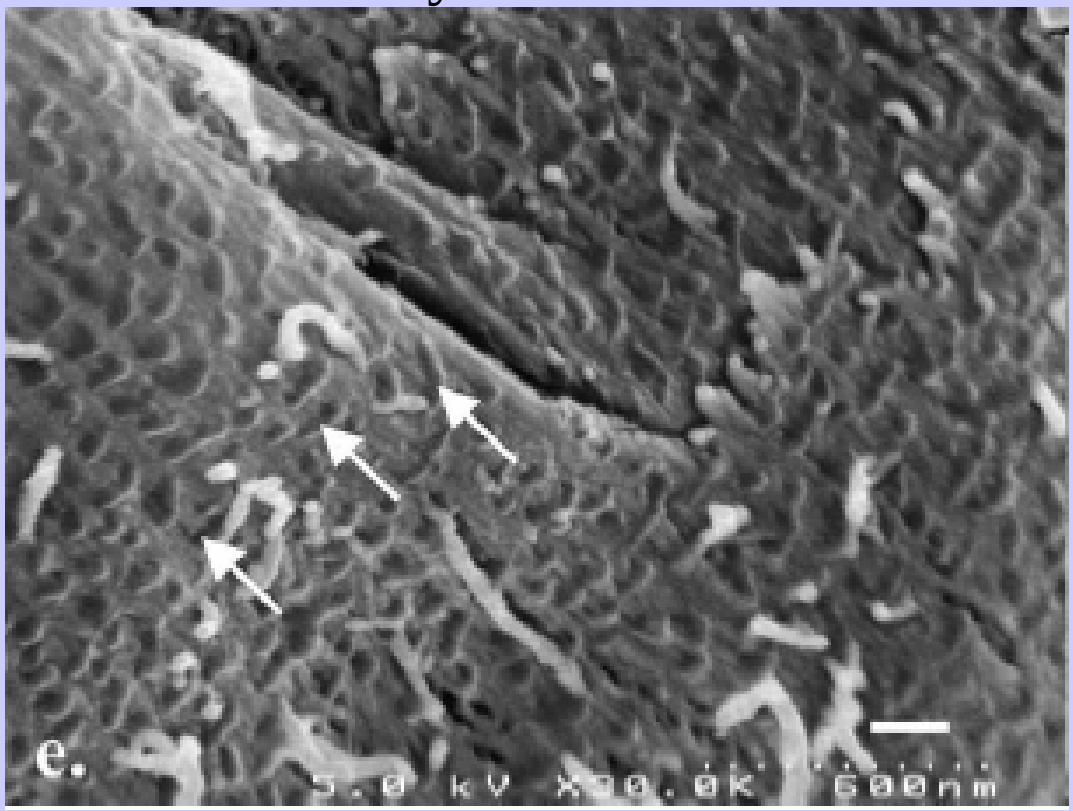
High resolution SEM with backscatter detection highlights 10 and 20 nm colloidal gold particles, which has great potential to allow fiber chemistry to be correlated with nanostructure.



Field emission SEM, 8kV, immunolabeling of surface proteins of stem cells
L.Cui et al. (2004) *J. Histochem. Cytochem.* 52: 1447 - 1457

Other microscopic techniques that may be advantageously coupled with immunolabeling.

Cryo-FE-SEM



Birch fiber during degradation by white rot fungus.

G. Daniel et al. (2004) *C.R. Biologie* 327: 861-871

Atomic Force Microscopy

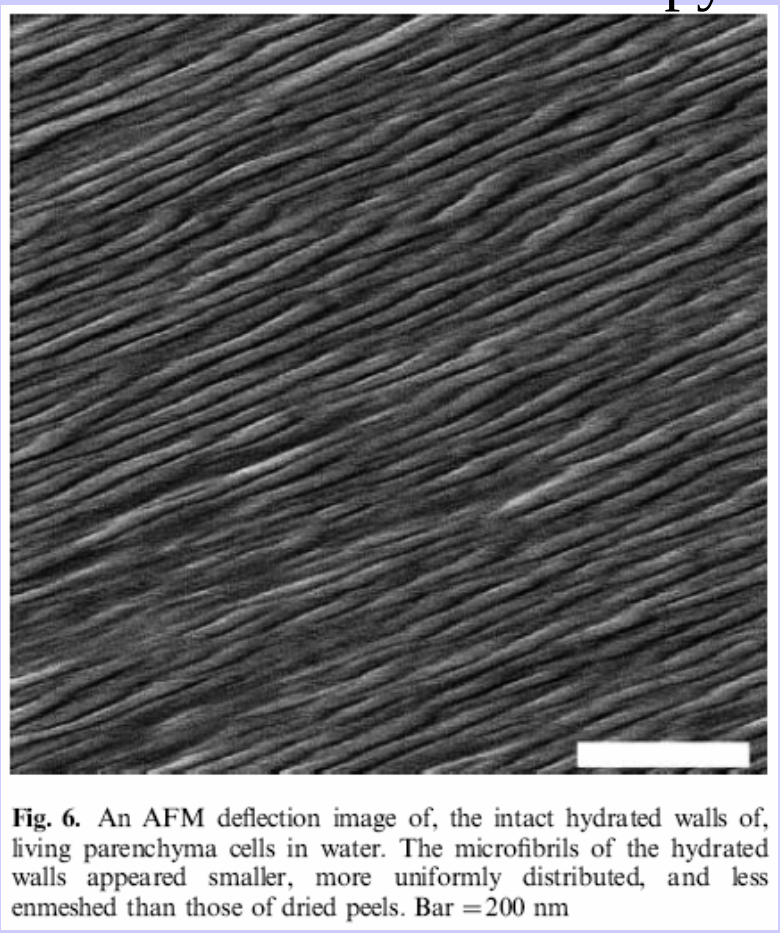


Fig. 6. An AFM deflection image of, the intact hydrated walls of, living parenchyma cells in water. The microfibrils of the hydrated walls appeared smaller, more uniformly distributed, and less enmeshed than those of dried peels. Bar = 200 nm

J.C. Thimm et al. (2000) *Planta* 212: 25-32

Use of immunolabeling with fibers and pulp has paralleled the availability of specific antibodies. Therefore, efforts to raise additional antibodies will lead to additional progress.

One such effort in relation to general cell wall oligo- and polysaccharides is in progress under the direction of Dr. Michael Hahn: http://cell.ccrc.uga.edu/~mao/wallmab/Home/Home.php

Monoclonal Antibodies for Plant Cell Walls

The University of Georgia

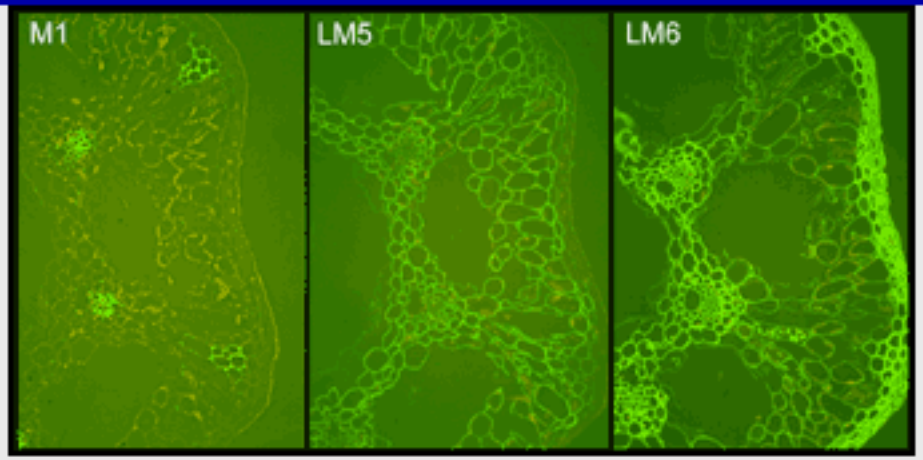
Home | People | Background | Research Plan | Wall Antigens | Antibodies | Facilities | Links

A NSF-funded Project to:

Generate monoclonal antibodies that recognize carbohydrate structures within plant cell walls.

Characterize the epitope structures recognized by these antibodies

Make the monoclonal antibodies available to the scientific community



Horsetail stem labeled with monoclonal antibodies that recognize:

Fucosylated xyloglucan (CCRC-M1)

1,4 linked galactan (LM5)

1,5-linked arabinan (LM6)

News:
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