A DNA based method for directed self assembly of cellulose nanocrystals into advanced nanomaterials

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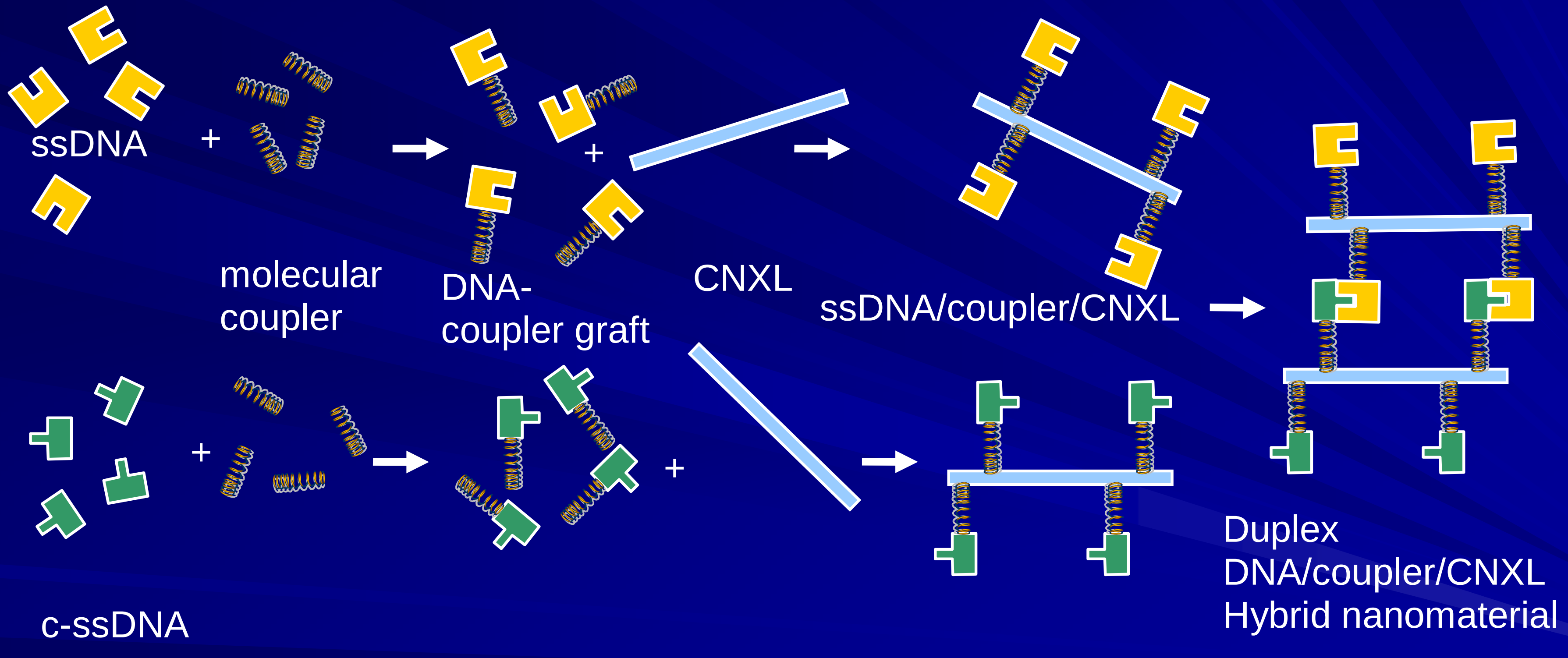
Outline

- Concept
- Background
- The experimental results
  - preliminary study
- Conclusions

Application = tissue engineering

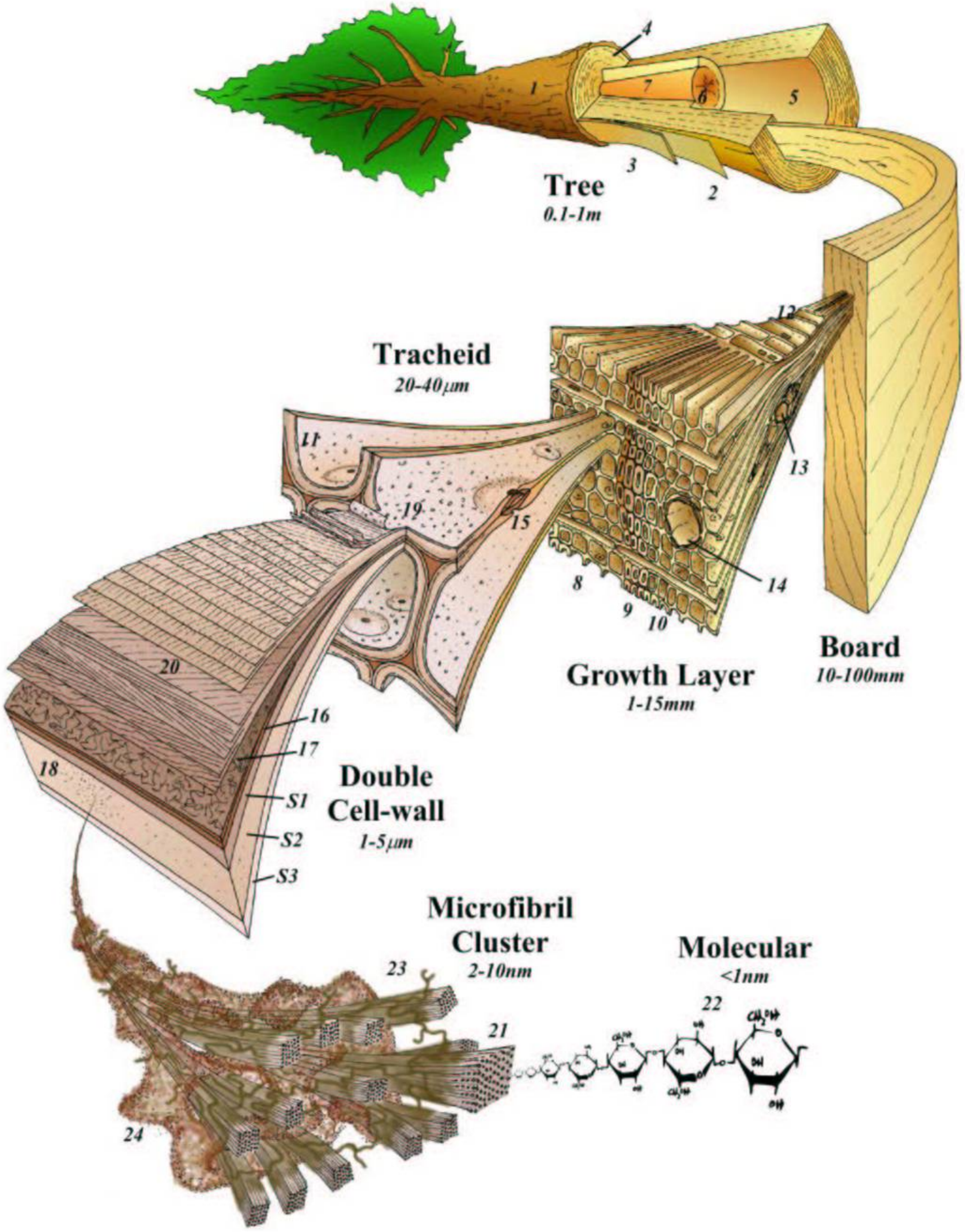
- A need exists for biomedical implants that can match the mechanical properties of the tissue, then dissolve in the body at a predetermined rate to match the body's regeneration of the tissue, e.g. bone, skin

concept


ssDNA
+
molecular
coupler
DNA-
coupler graft
+
CNXL
ssDNA/coupler/CNXL
+
+
+
c-ssDNA
Duplex
DNA/coupler/CNXL
Hybrid nanomaterial

SOURCES OF:
CELLULOSE NANOCRYSTALS

Wood

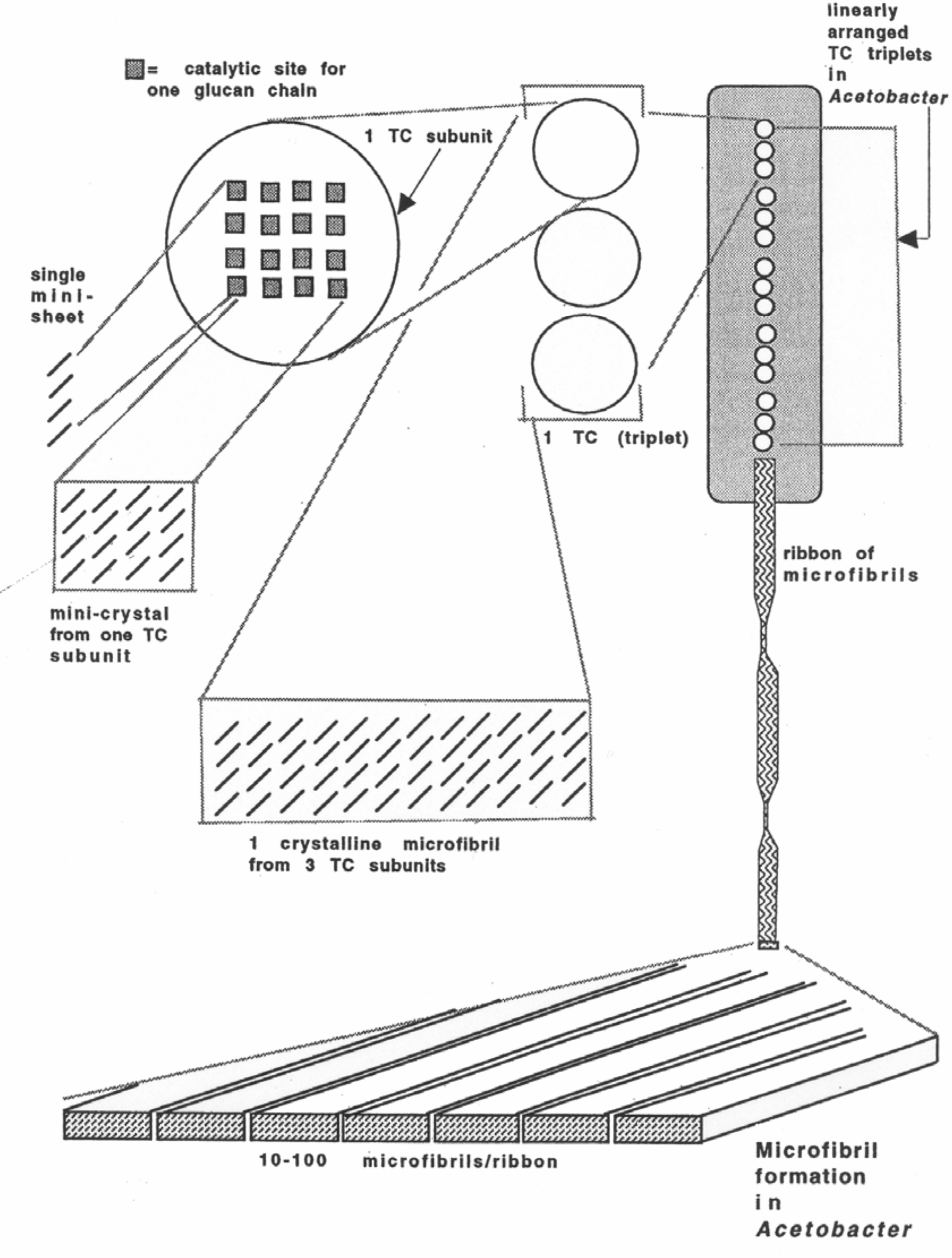

Tree
0.1-1m
Board
10-100mm
Growth Layer
1-15mm
Tracheid
20-40µm
Double
Cell-wall
1-5µm
S1
S2
S3
Microfibril
Cluster
2-10nm
Molecular
<1nm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

Sugar Beets

Cotton

Barnacles (tunicin)

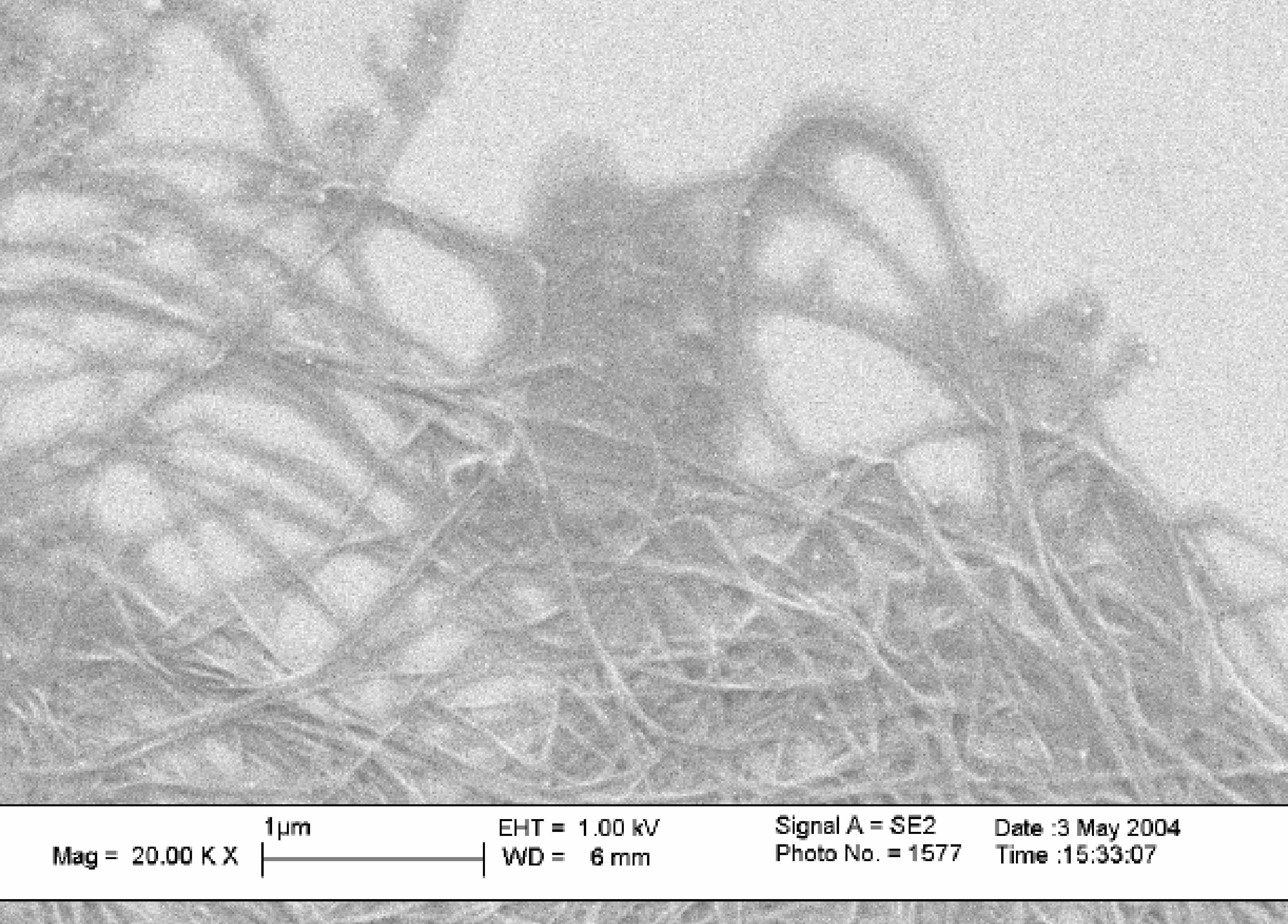
Bacterial Cellulose



CELLULOSE BIOSYNTHESIS

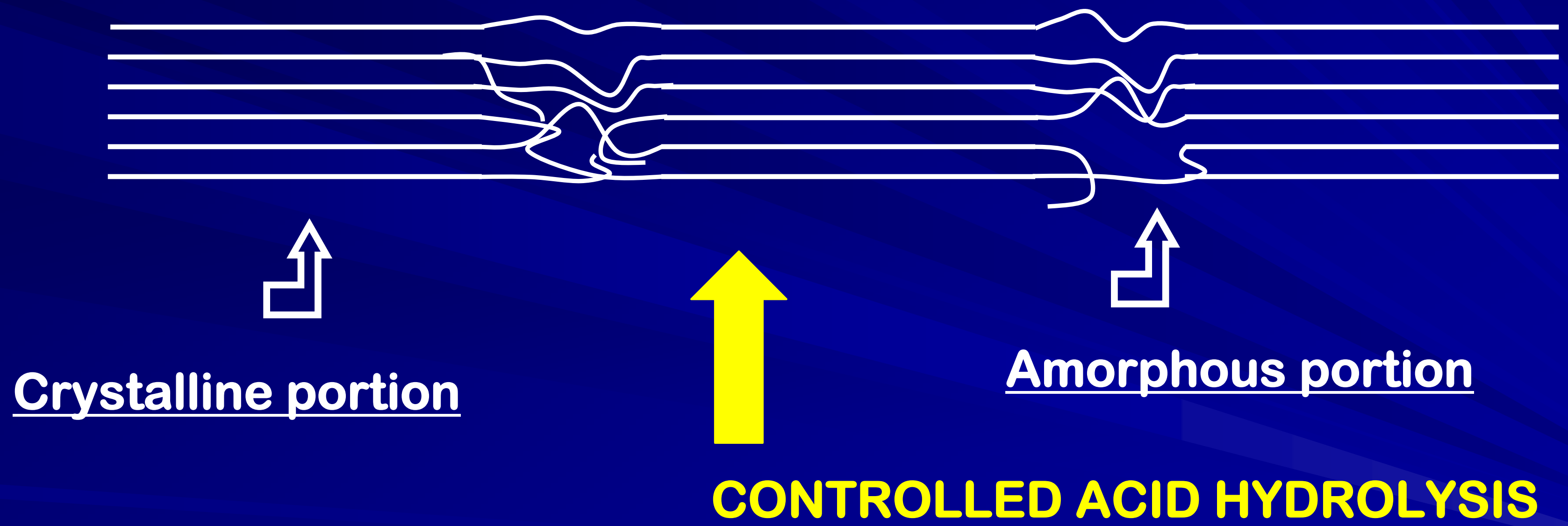
R.M. Brown, 1996. J. Mat. Sci. – Pure Appl. Chem. A33(10): 1345-1373

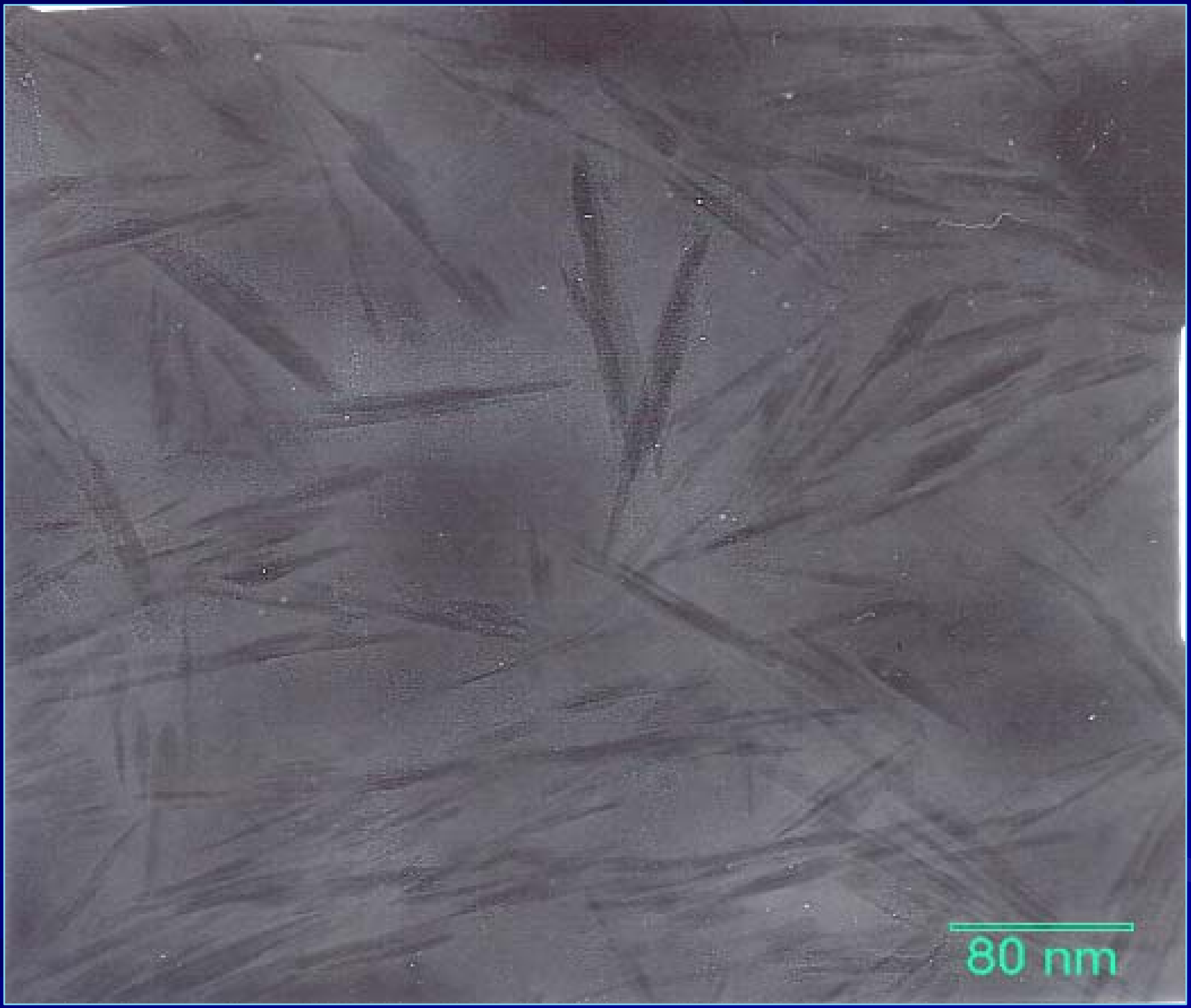
Slide from Wankei Wan, U. W. Ontario, London, ON, Canada



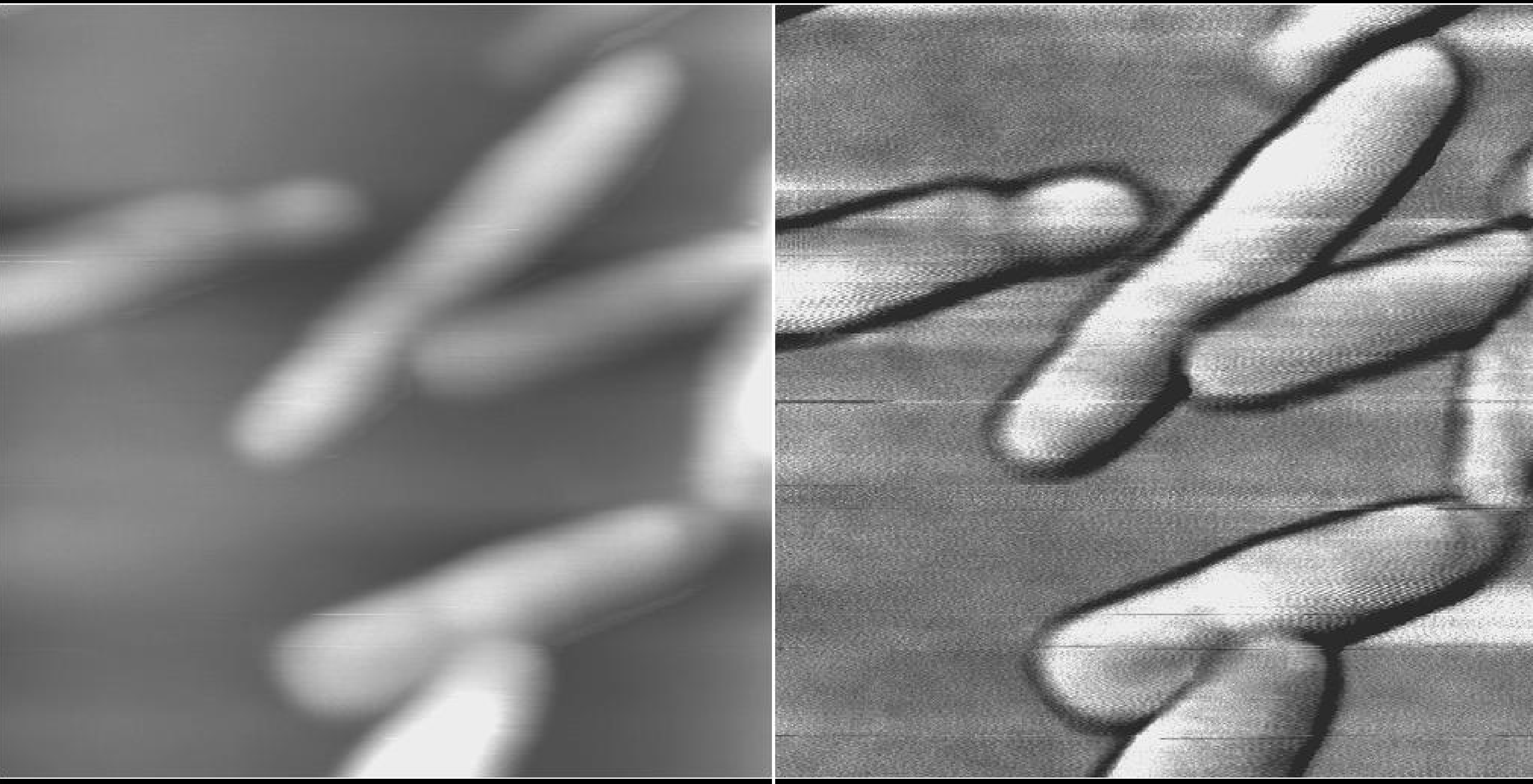
CELLULOSE NANOCRYSTAL PRODUCTION

- Native cellulose - Semi crystalline Polymer (~70% crystalline).





TEM image of cellulose nanocrystals

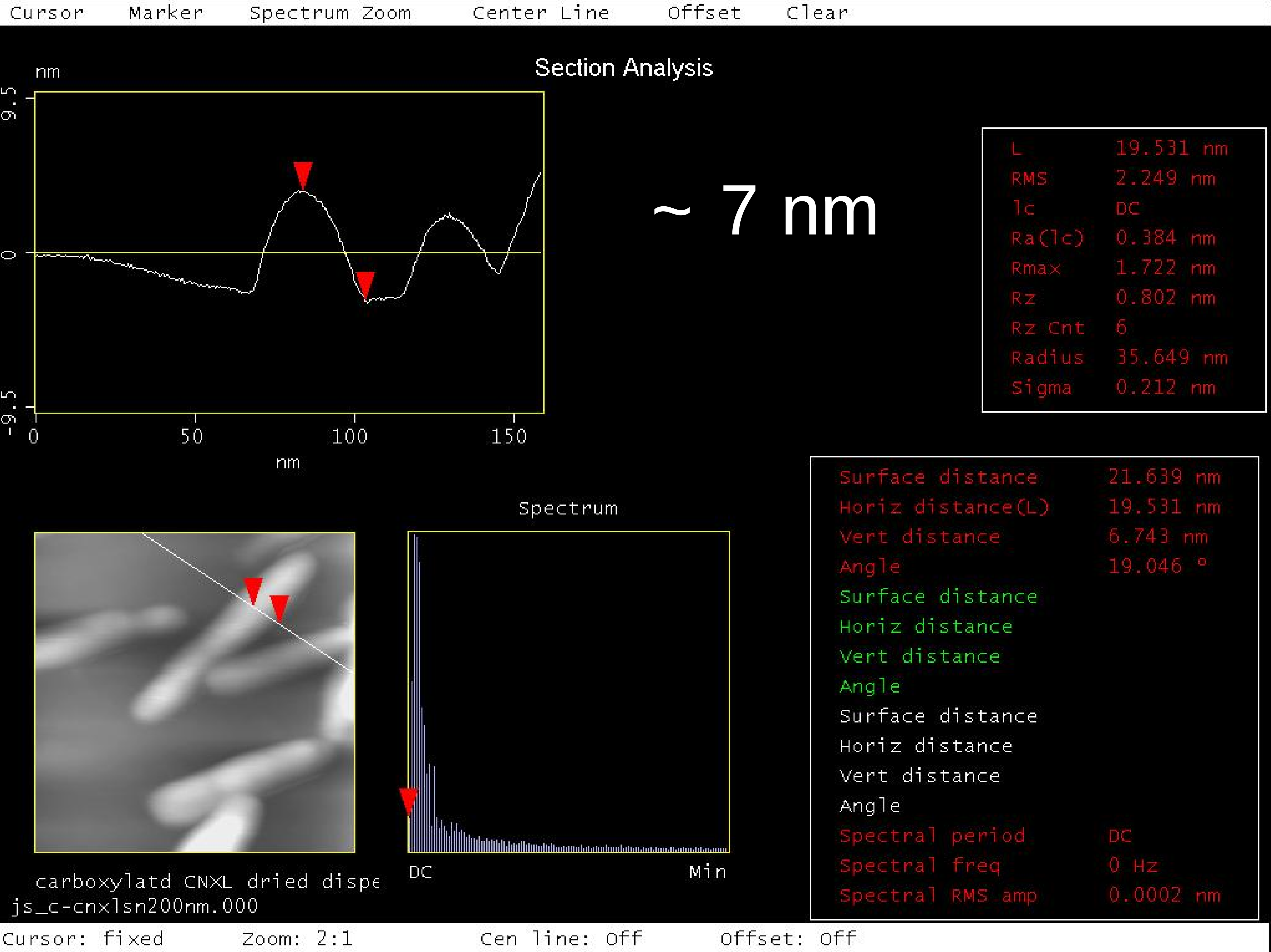


0 200 nm 0 200 nm

Data type Height
Z range 19.68 nm

Data type Phase
Z range 28.11 °

js_c-cnxlsn200nm.000
carboxylatd CNXL dried dispersion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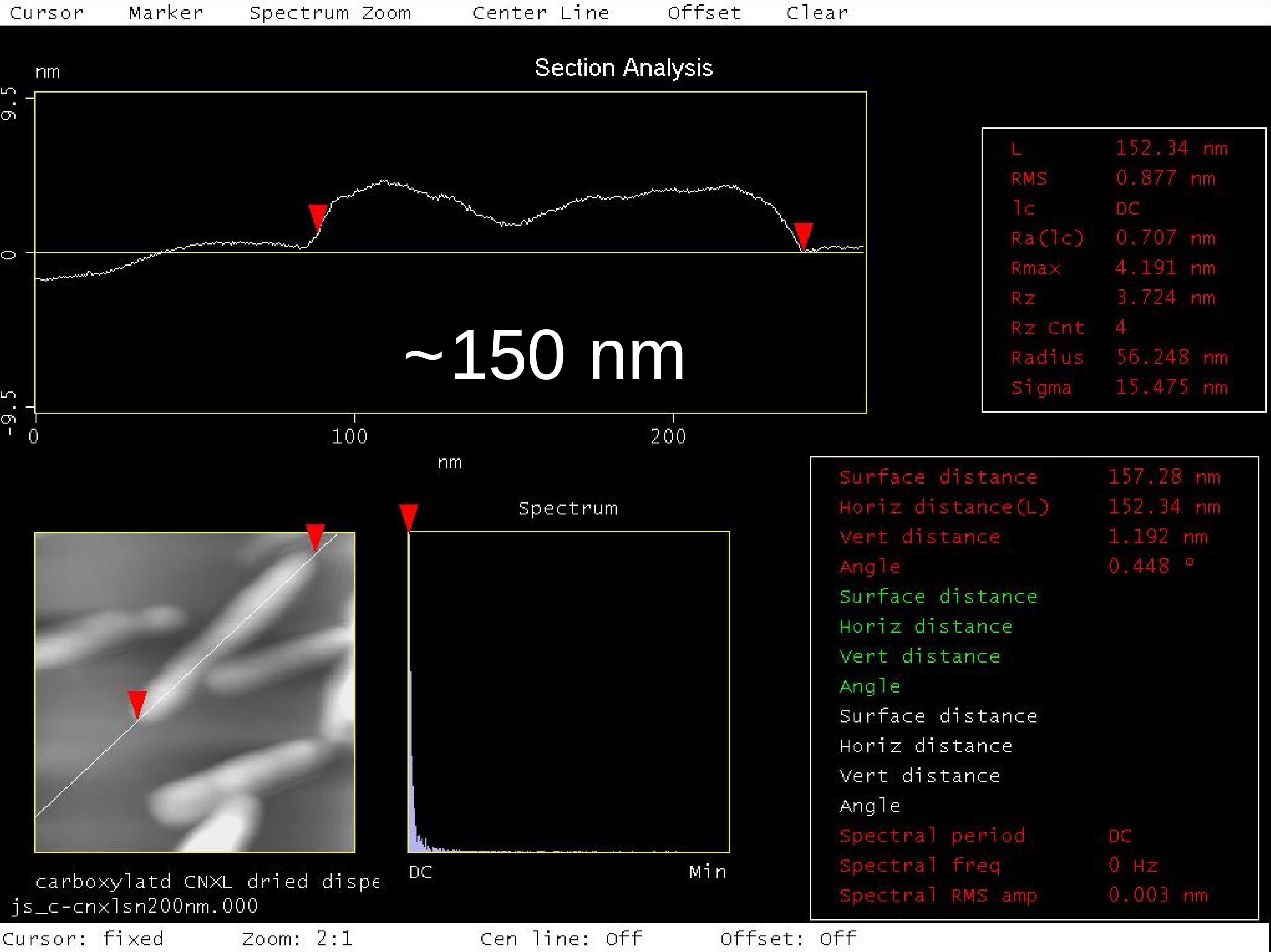
Section Analysis
nm
9.5
0
-9.5
0
50
100
150
nm
~ 7 nm
L 19.531 nm
RMS 2.249 nm
lc DC
Ra(lc) 0.384 nm
Rmax 1.722 nm
Rz 0.802 nm
Rz Cnt 6
Radius 35.649 nm
Sigma 0.212 nm
Spectrum
DC
Min
carboxylatd CNXL dried dispe
js_c-cnxlsn200nm.000
Surface distance 21.639 nm
Horiz distance(L) 19.531 nm
Vert distance 6.743 nm
Angle 19.046 °
Surface distance
Horiz distance
Vert distance
Angle
Surface distance
Horiz distance
Vert distance
Angle
Spectral period DC
Spectral freq 0 Hz
Spectral RMS amp 0.0002 nm
Cursor: fixed Zoom: 2:1 Cen line: Off Offset: Off


Cursor Marker Spectrum Zoom Center Line Offset Clear
Section Analysis
nm
9.5
0
-9.5
0
100
200
nm
~150 nm
L 152.34 nm
RMS 0.877 nm
lc DC
Ra(lc) 0.707 nm
Rmax 4.191 nm
Rz 3.724 nm
Rz Cnt 4
Radius 56.248 nm
Sigma 15.475 nm
Spectrum
DC
Min
Surface distance 157.28 nm
Horiz distance(L) 152.34 nm
Vert distance 1.192 nm
Angle 0.448 °
Surface distance
Horiz distance
Vert distance
Angle
Surface distance
Horiz distance
Vert distance
Angle
Spectral period DC
Spectral freq 0 Hz
Spectral RMS amp 0.003 nm
carboxylatd CNXL dried dispe
js_c-cnxlsn200nm.000
Cursor: fixed
Zoom: 2:1
Cen line: Off
Offset: Off

CELLULOSE NANOCRYSTALS

Cellulose source	Length	Cross section	Aspect ratio
Tunicate	100 nm – microns	10-20 nm	5 to > 100 (high)
Algal (Valonia)	> 1000 nm	10 to 20 nm	50 to > 10 nm (high)
Bacterial	100 nm – microns	5-10 x 30-50 nm	2 to > 100 (medium)
Cotton	200-350 nm	5 nm	20 to 70 (low)
Wood	100–300 nm	3 – 5 nm	20 to 50 (low)

Beck-Candanedo, et. al. Biomacromol. (2005) 6:1048-1054

Surface Area

	m^2/g
E-glass fibers*	**~1**
Paper fibers	**4**
Graphite	**25-300**
Fumed silica	**100-400**
Fully exfoliated clay	**~ 500**
Cellulose nanocrystals**	**250**
Carbon nanotubes***	**~ 100 - ?**

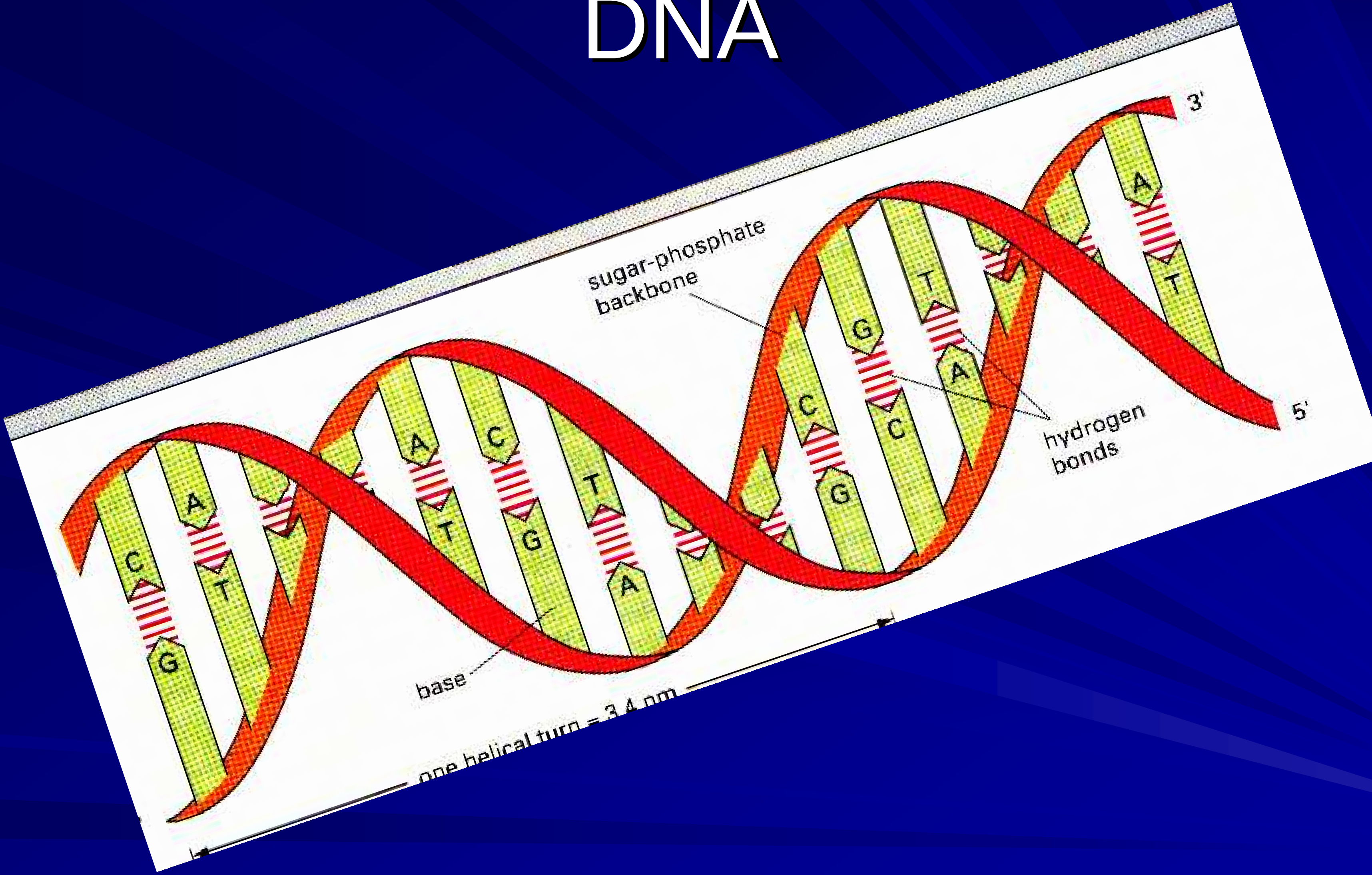
*http://www.jm.com/engineered_products/filtration/products/microfiber.pdf
** Winter, W. presentation at ACS meeting, San Diego, March 2005
***http://www.ipme.ru/e-journals/RAMS/no_5503/staszczuk/staszczuk.pdf.

MECHANICAL PROPERTIES

Material	Tensile strength GPa	Modulus GPa
cellulose crystal	7.5[1]	145[2]
Glass fiber	4.8	86
Steel wire	4.1	207
Graphite whisker	21	410
Carbon nanotubes[3]	11-63	270-970

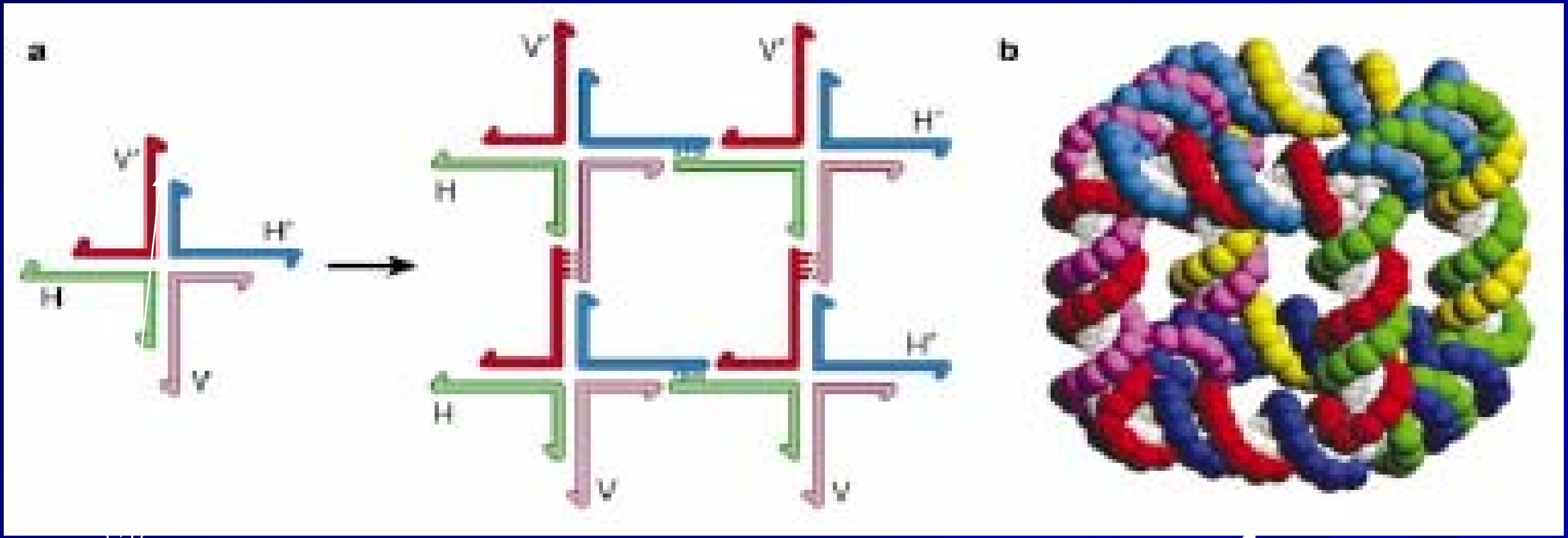
1. Marks, *Cell wall mechanics of tracheids* **1967**
2. **Sturcova, et al. (2005) Biomacromol. 6, 1055**
3. **Yu, et al *Science* (2000) *287*, 637**

DNA



http://tigger.uic.edu/classes/phys/phys461/phys450/ANJUM04/DNA_helix.jpg

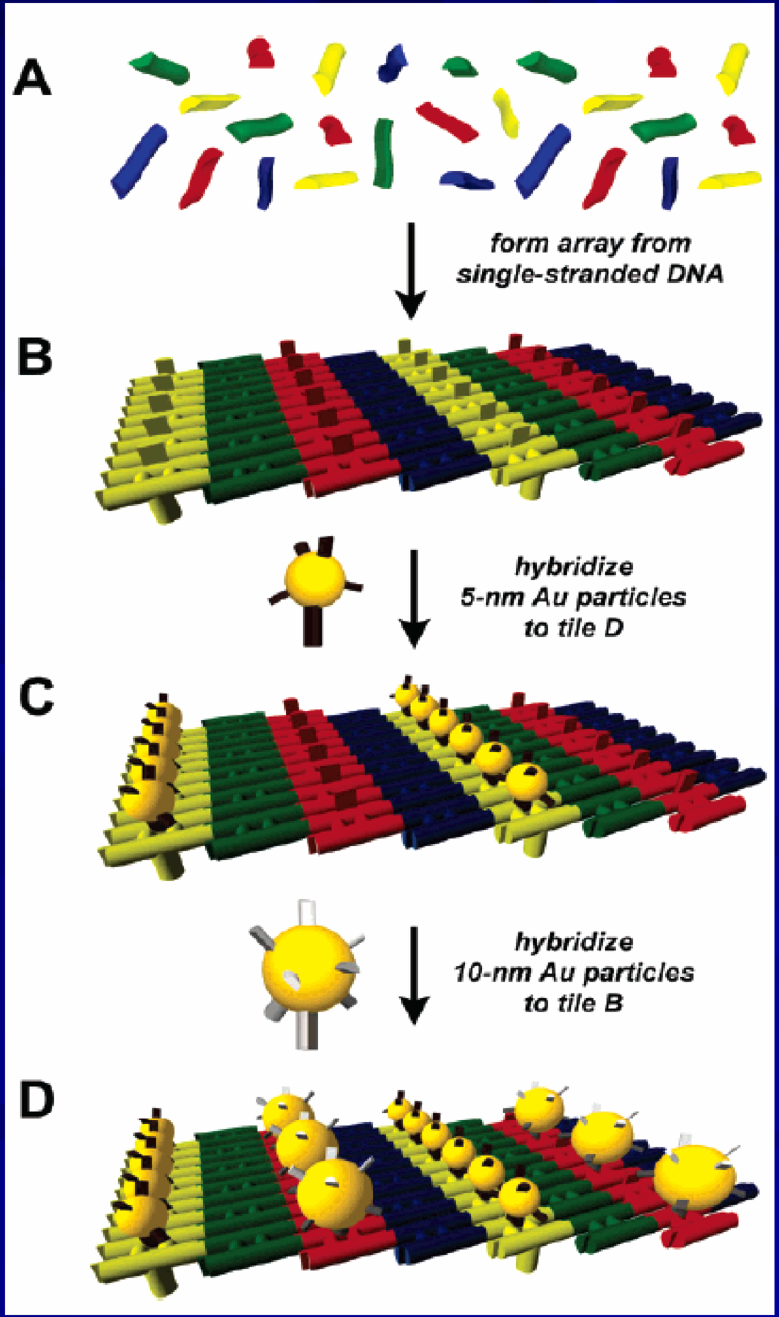
Branched structures



Sticky ends

cube

Seeman 2003


A
form array from
single-stranded DNA
B
hybridize
5-nm Au particles
to tile D
C
hybridize
10-nm Au particles
to tile B
D

Pinto 2005

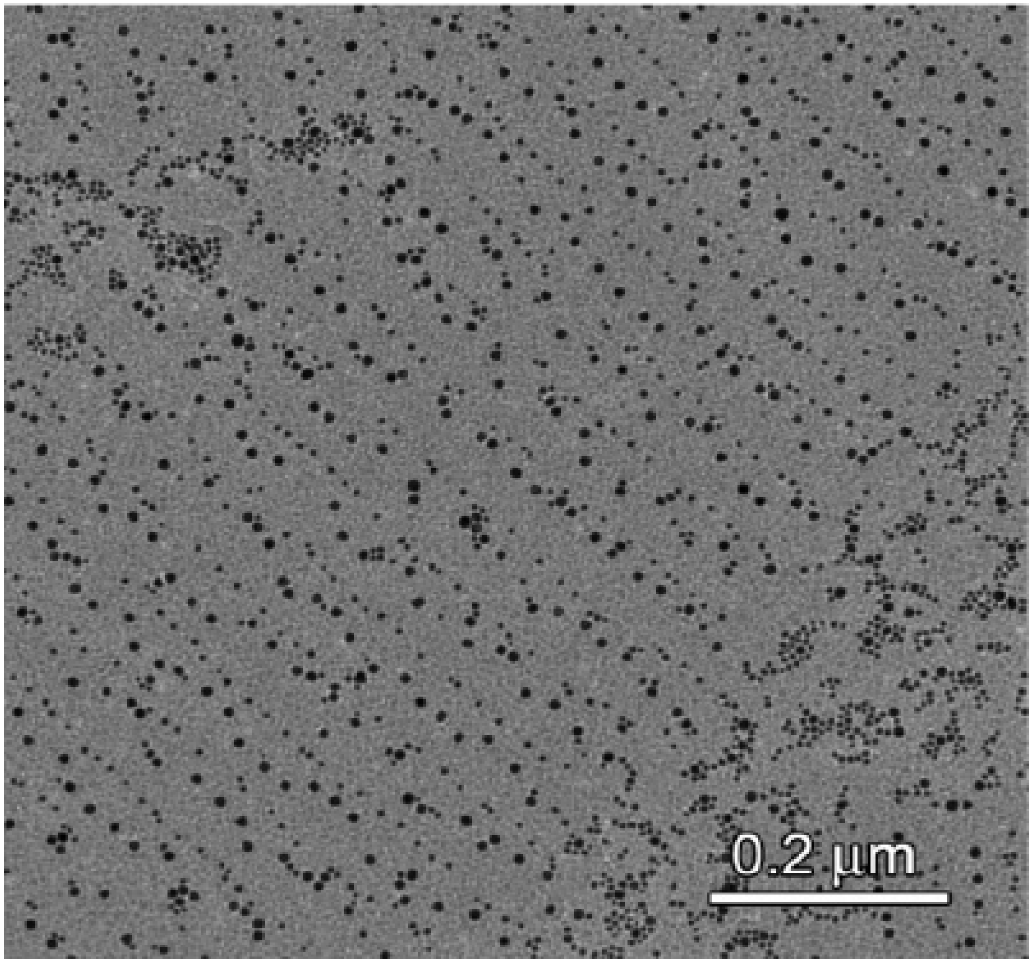


Figure 4. TEM image of the two-particle array. The pattern of alternating parallel rows of small and large gold particles is clearly visible. (Particles tend to aggregate on the mica surface outside the arrays, e.g., lower right corner of image.)

Pinto 2005

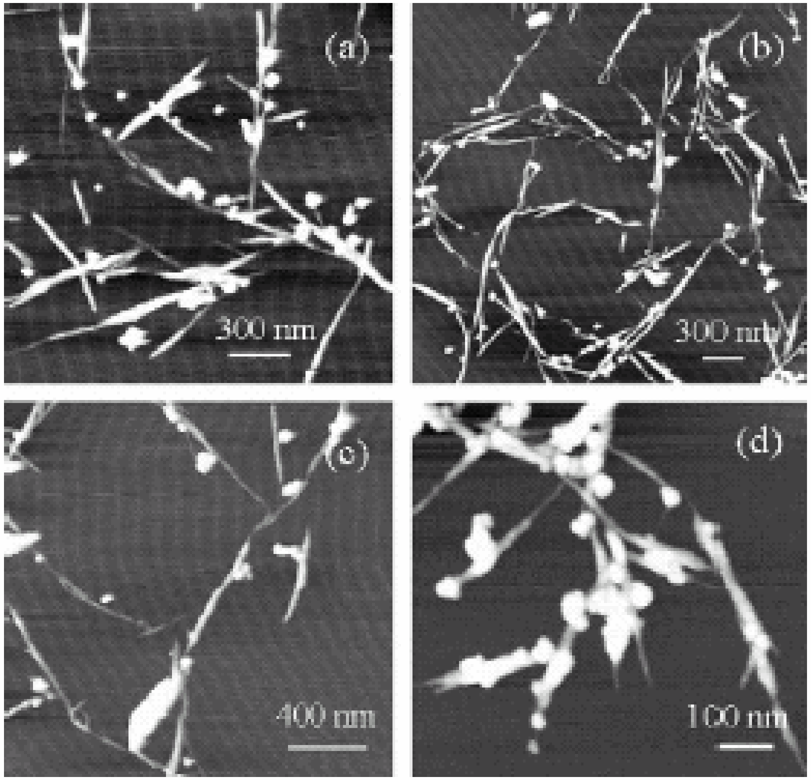


Fig. 1. AFM images of self-assembled SWNTs via hybridization of DNA, (a)–(d) are typical images from four different AFM samples.

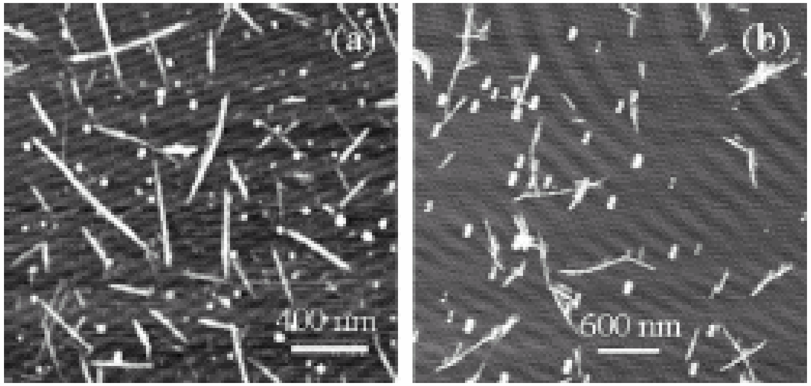


Fig. 2. Typical AFM images of unfunctionalized SWNTs (a) and ssDNA-SWNTs (b).

Y. Lu et al. / Chemical Physics Letters 419 (2006) 390–393

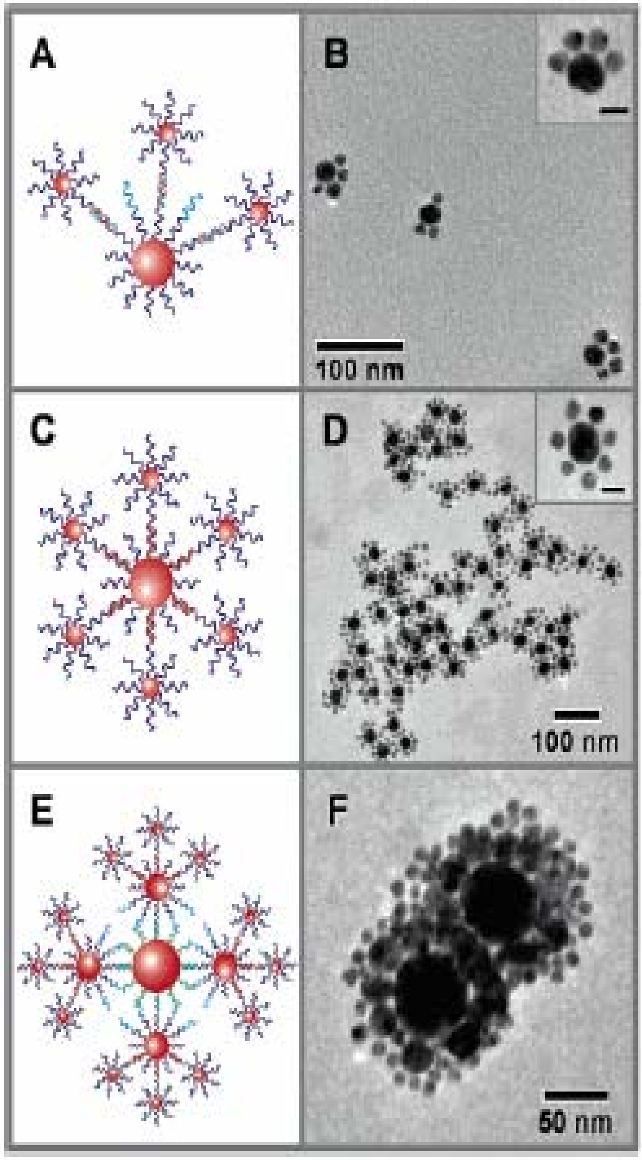


Figure 2. Directional assembly of asymmetrically functionalized AuNPs into (A, B) cat paw, (C, D) satellite, and (E, F) dendrimer-like structures. Inset: scale bar = 20 nm.

Xiaoyang Xu, Nathaniel L. Rosi, Yuhuang Wang, Fengwei Huo, and Chad A. Mirkin*

JACS 2006, 128, 9286-9287

CNXL-DNA experiment

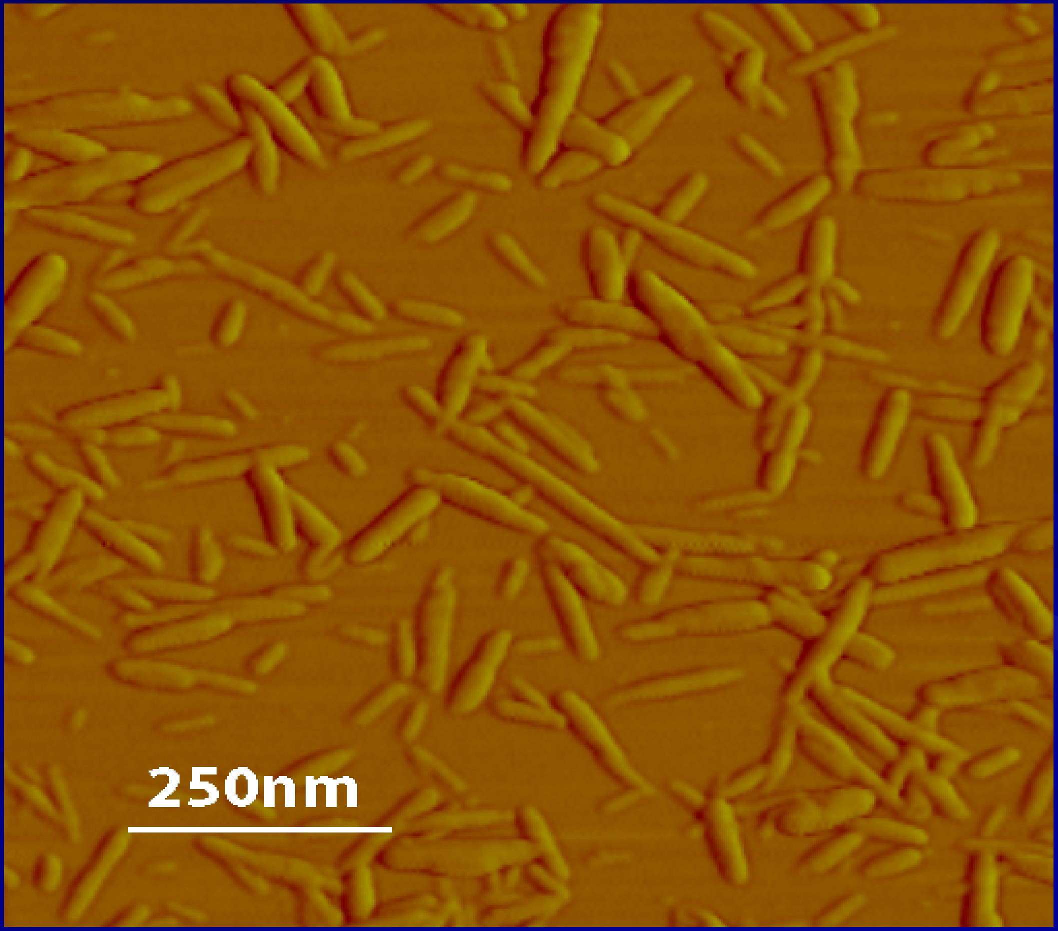
Surface modification of CNXLs

CNXLs C.CNXLs

- Titration of C.CNXLs indicated the presence of 1.4 mmols of acid/ g CNXLs.

Araki et.al, Langmuir, 17: 21-27, 2001

AFM of Carboxy-CNXLs



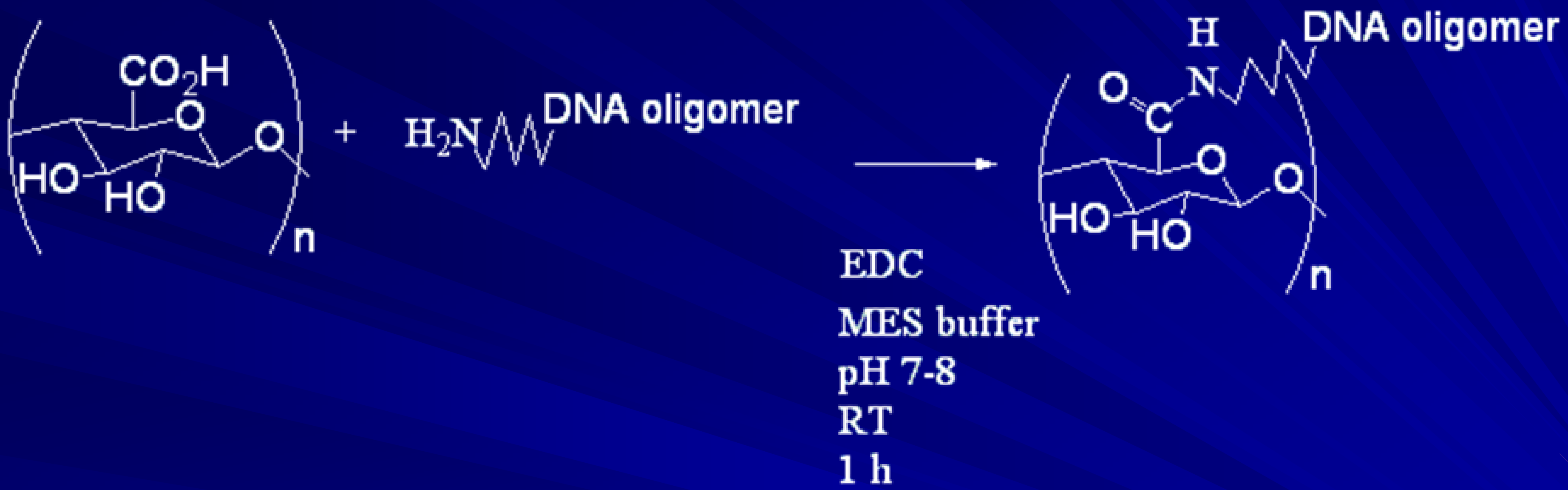
Oligomers

- Dodecyl linker:
  - 5'-amino-C12-CAGTCAGATCAGGACATGAGATCAT GCTAGTCAGCTACGGTCACTGCTAGTCCGTAC GTACCATGTCATAGTGTAGGT-3‘
  - And compliment
  - GC content = 49%
  - T_m = 70 °C
  - Purchased from IDT, Inc.

Oligomers

- Hexamer linker:
  - 5'-amino-C6-GCT CTA CCT GAC TAG CTC GT-3' and compliment
  - GC content = 55%
  - T_m = 56 °C
  - Purchased from Oligos, etc.

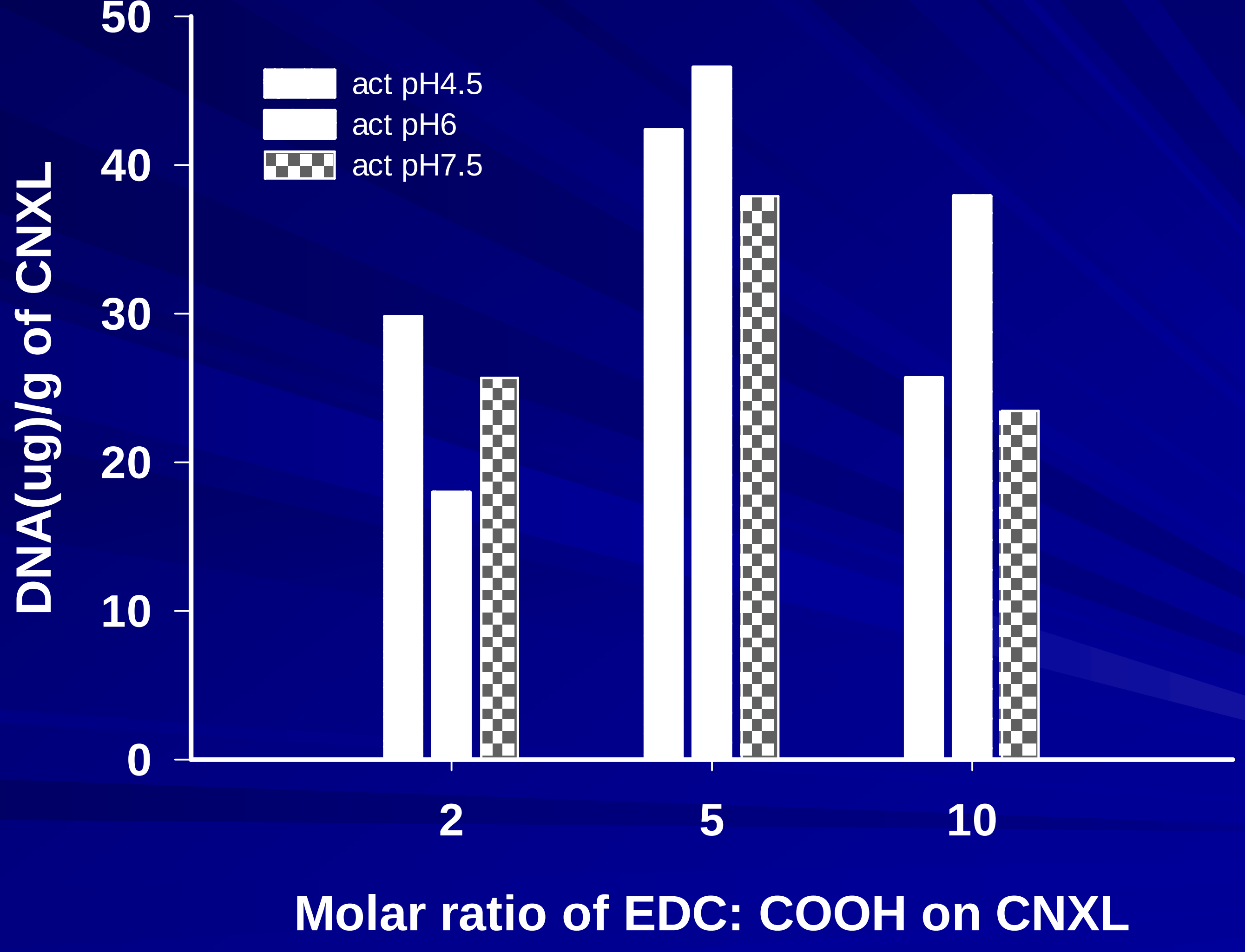
Classic EDC reaction



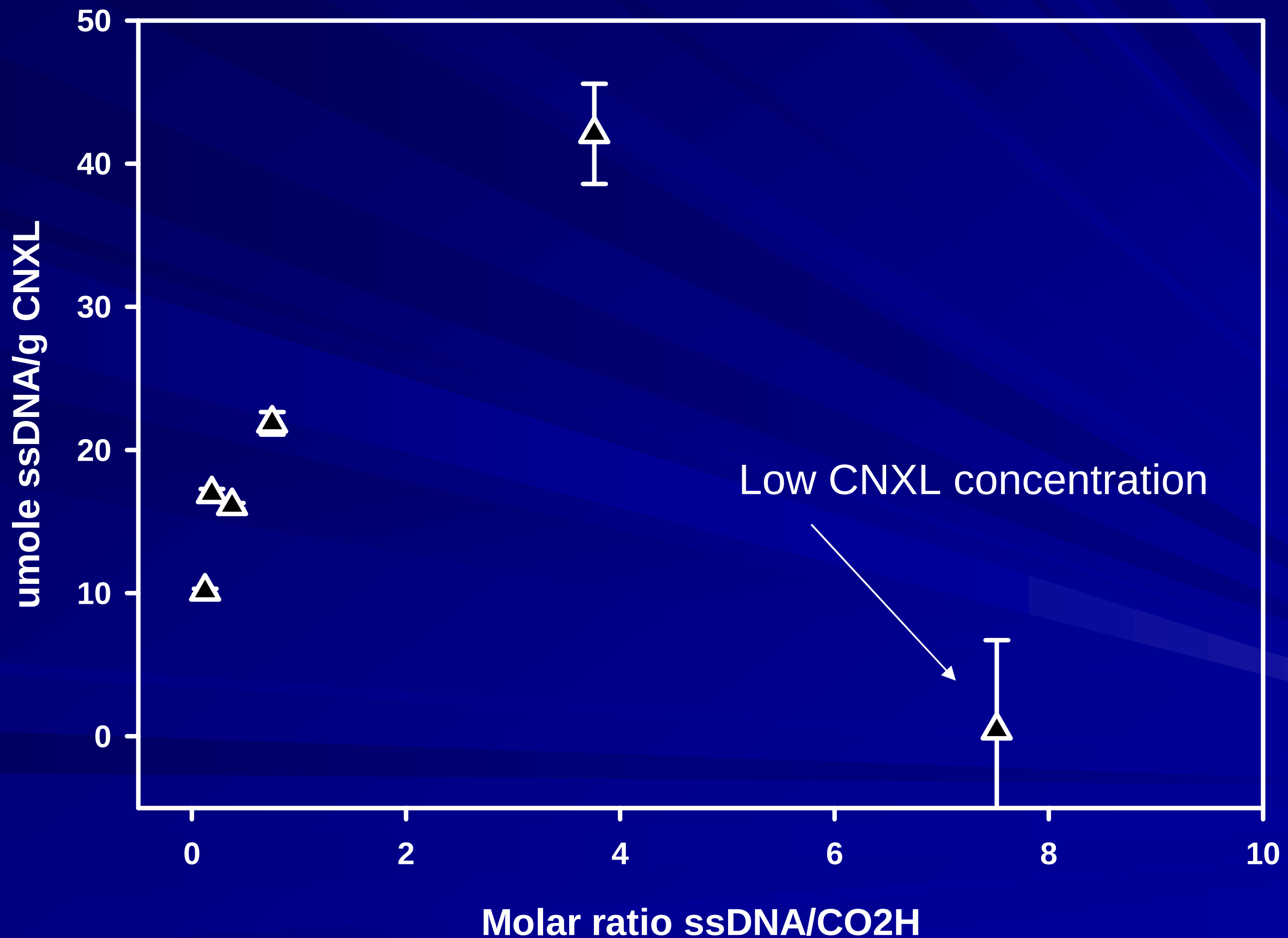
EDC = 1-Ethyl-3-[3-dimethylaminopropyl]**carbodiimide** Hydrochloride

Voicu 2004
Araki 2001
Deen 1990

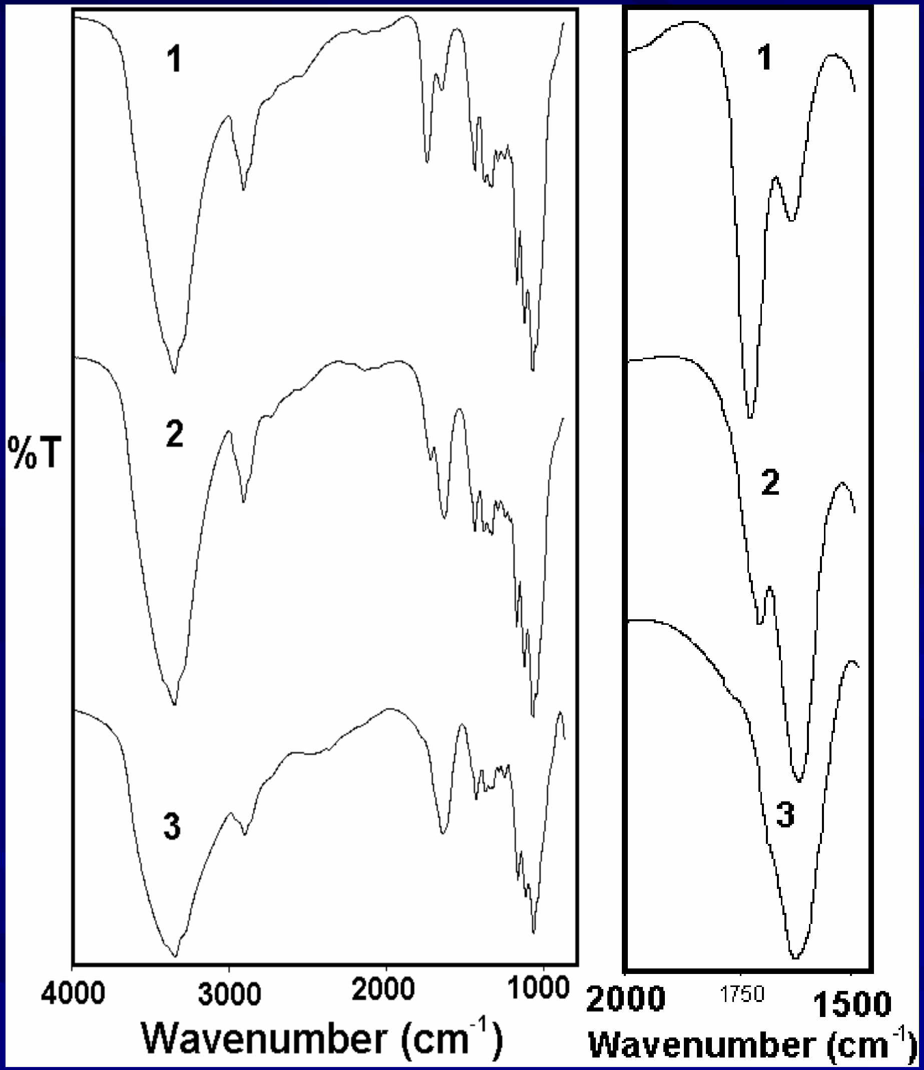
Optimization of grafting reaction EDC/CNXL ratio



Optimization of grafting reaction DNA/CNXL ratio



FTIR

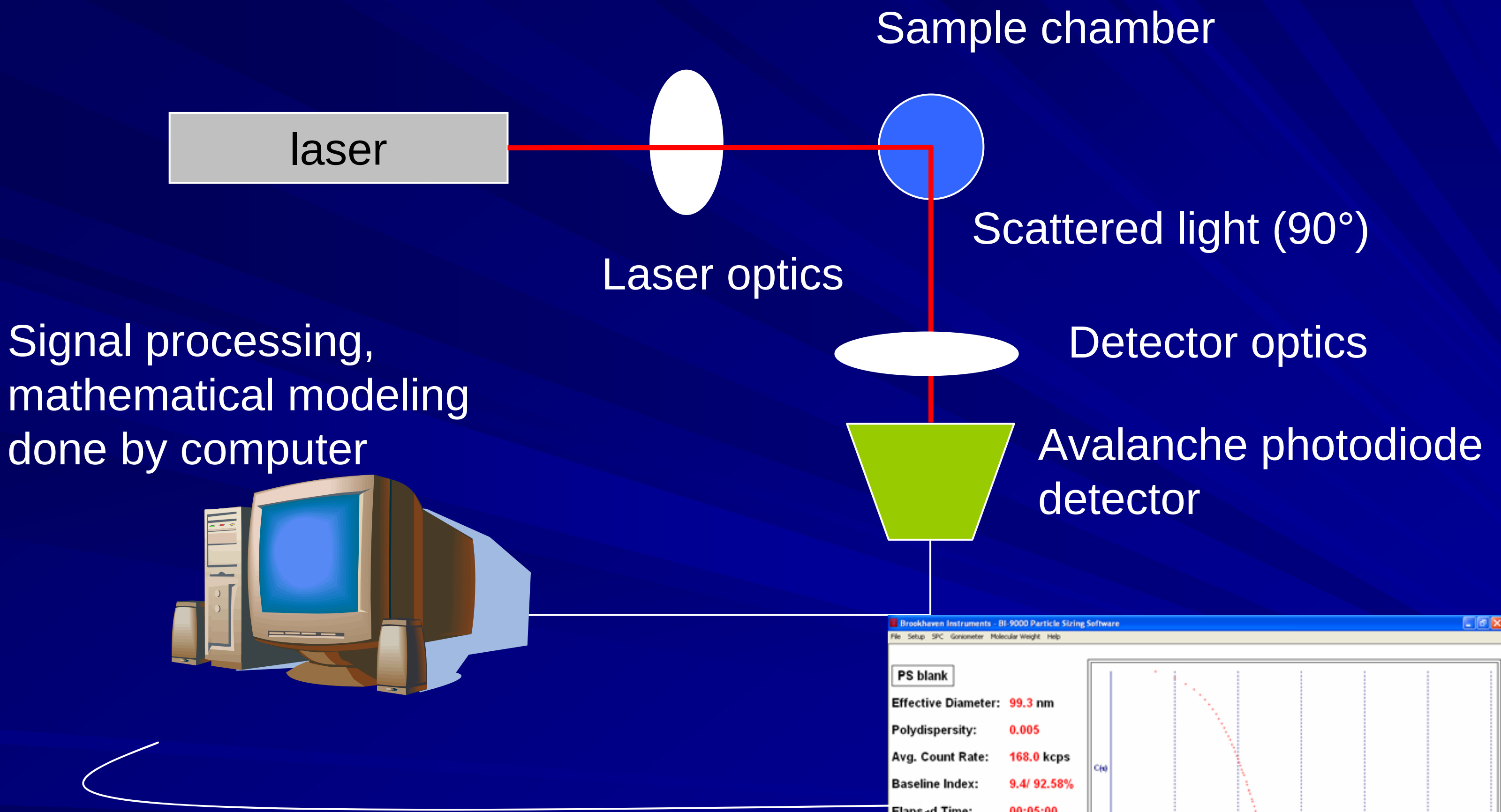


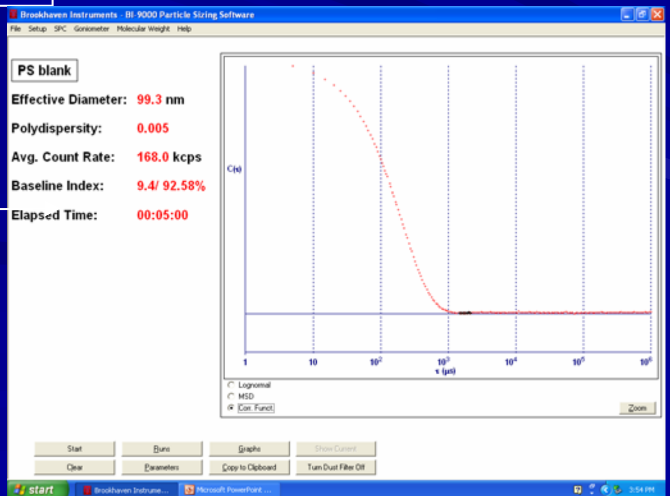
1. Carboxy-CNXL, protonated
2. EDC-NHS activated carboxy-CNXL, pH 4.5
3. pH 7.5

Mix the complimentary strands together

ssDNA-CNXL   Duplex DNA-CNXL   Carboxy-CNXL

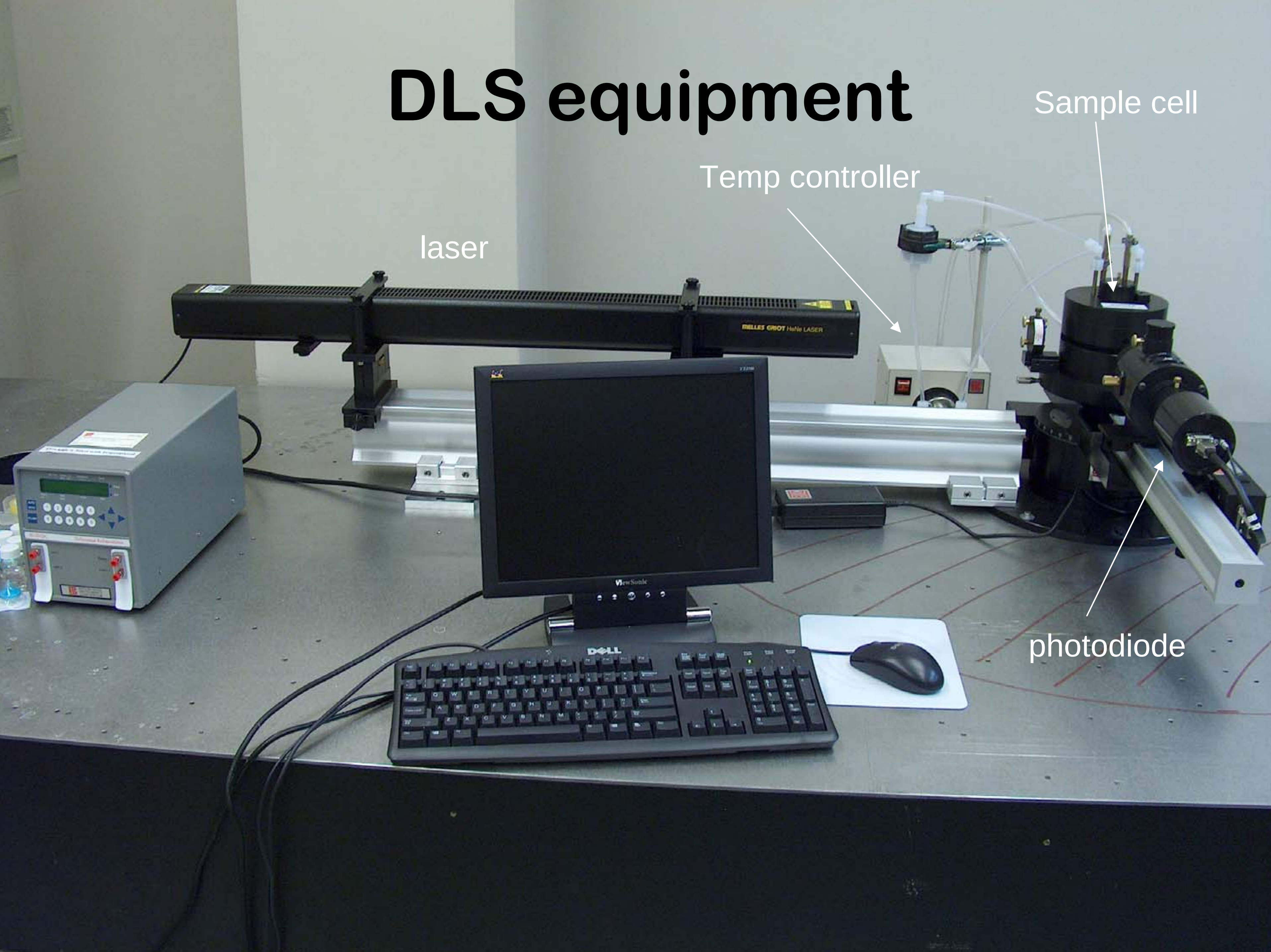
Dynamic light scattering (DLS)





- Spherical shape assumed by software

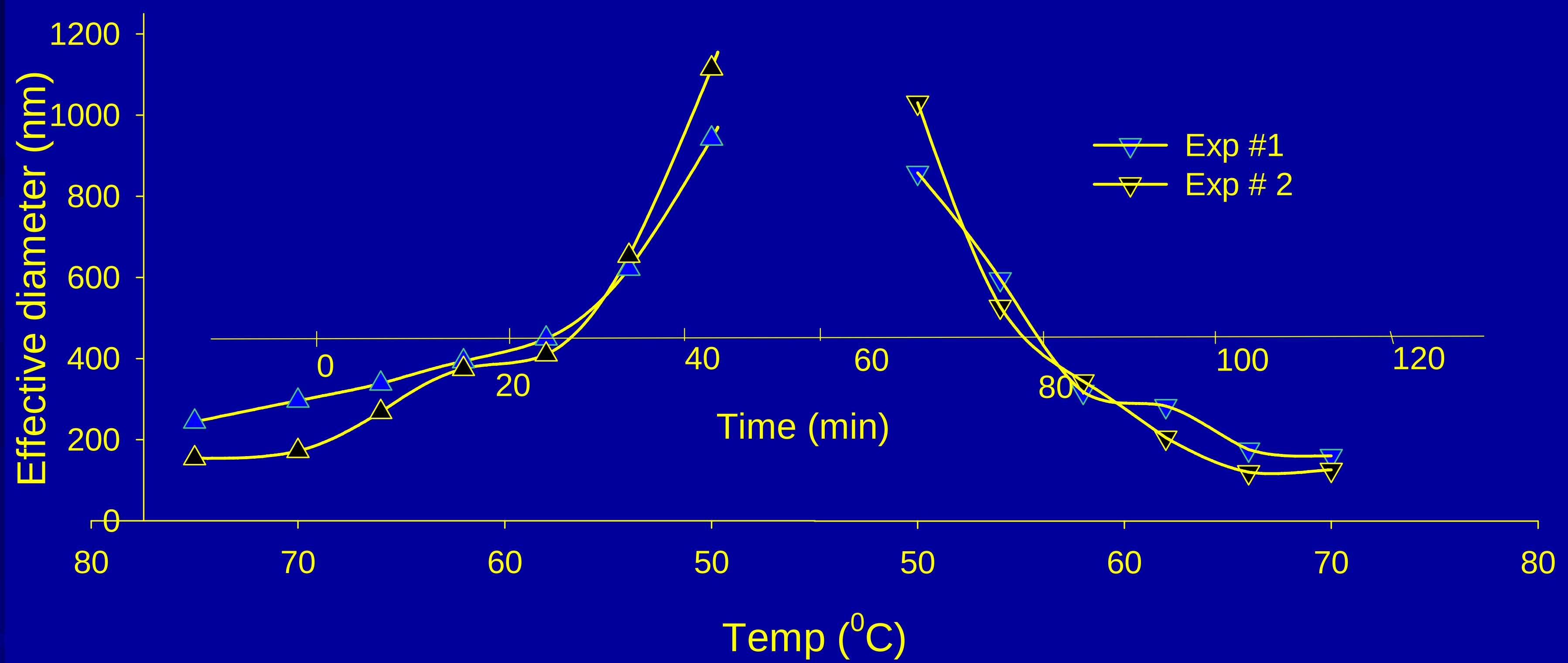
DLS equipment



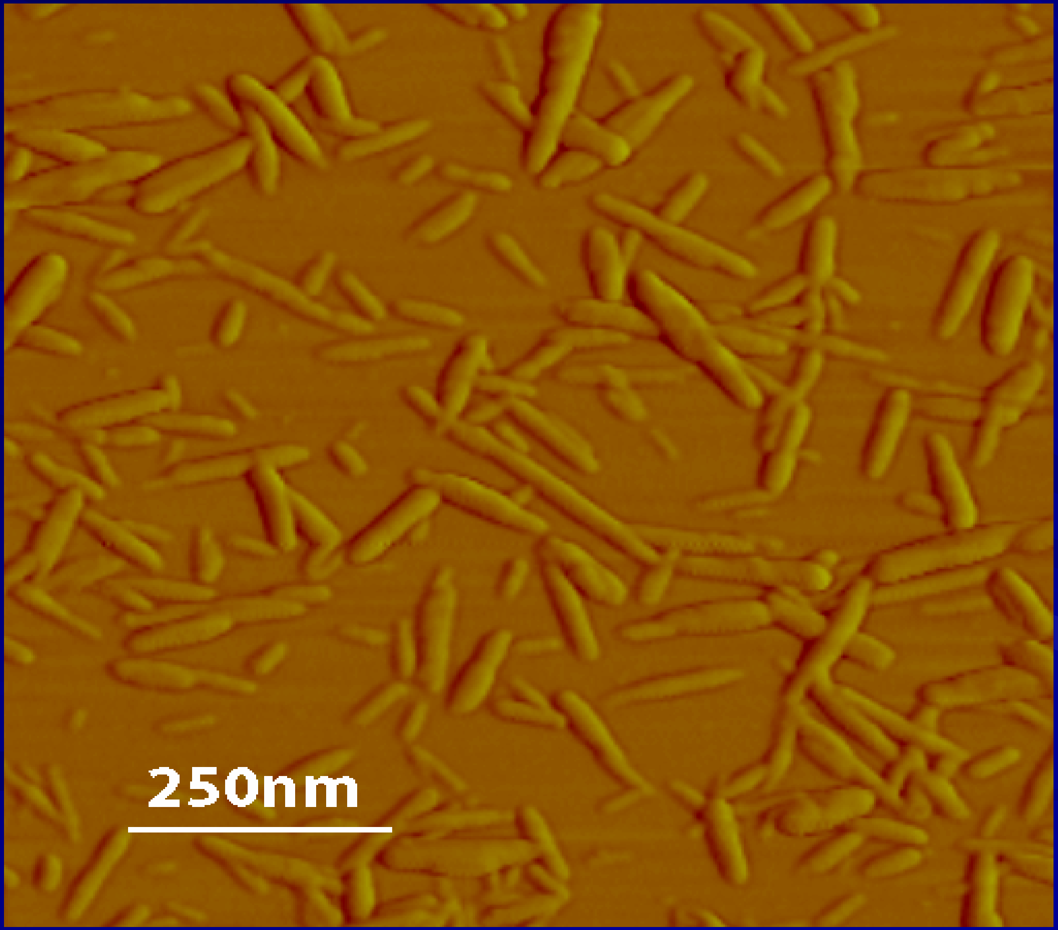
Dynamic light scattering results

Particle	**Diameter (D_h) at 25^oC**
CNXL	90-100 nm
Carboxy-CNXL	120-130 nm
ssDNA-CNXL	140-158 nm
dsDNA-CNXL duplex	548-620 nm

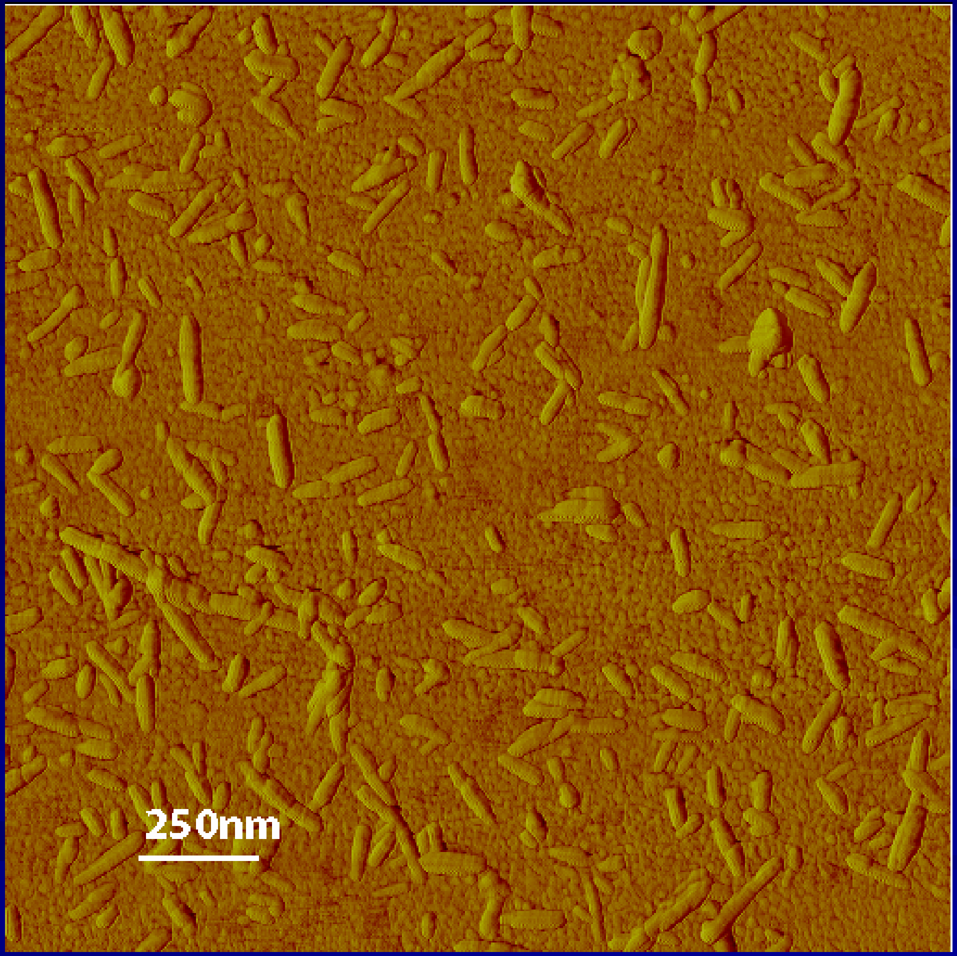
DLS temperature cycling experiment



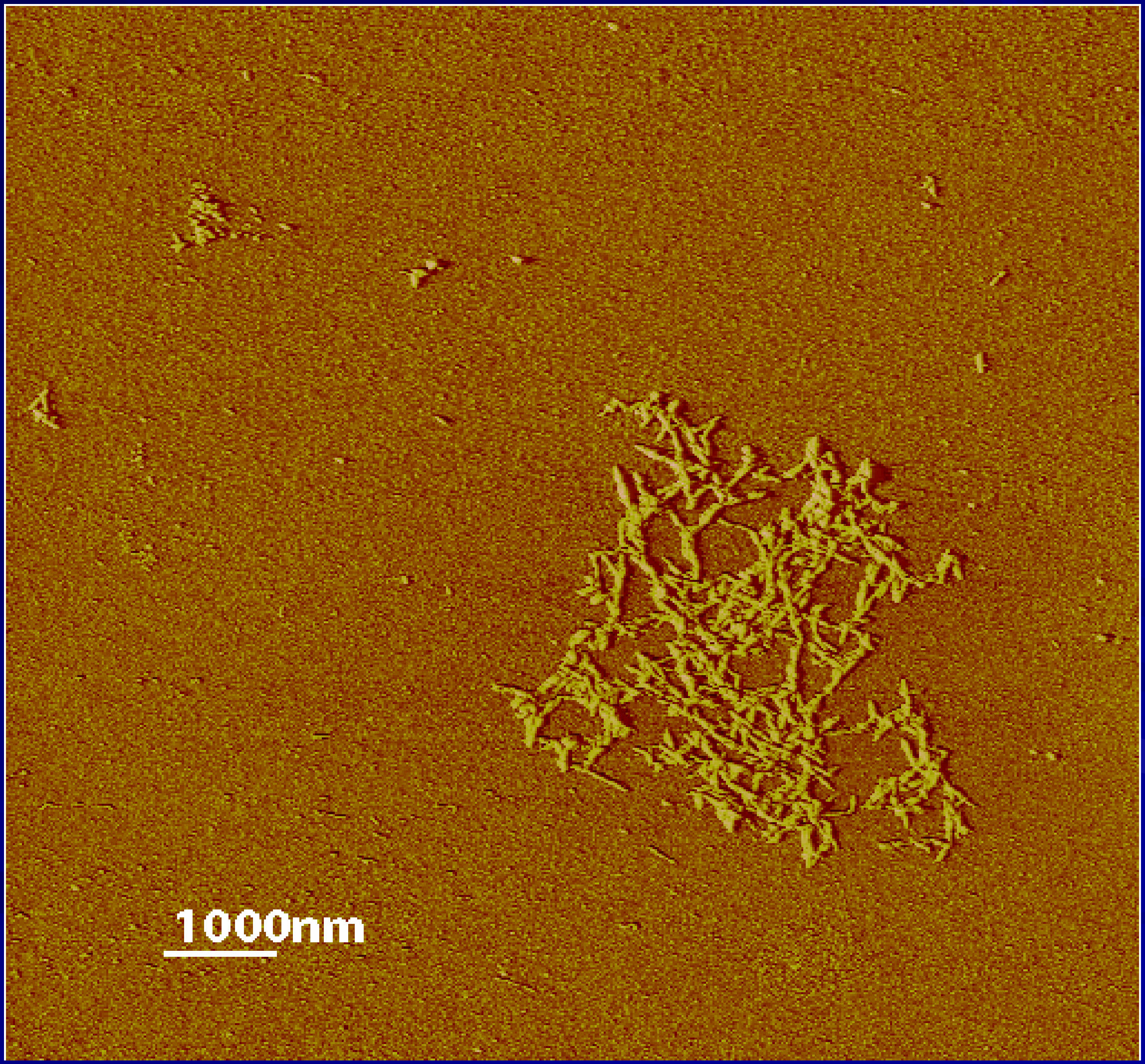
AFM of carboxy-CNXLs



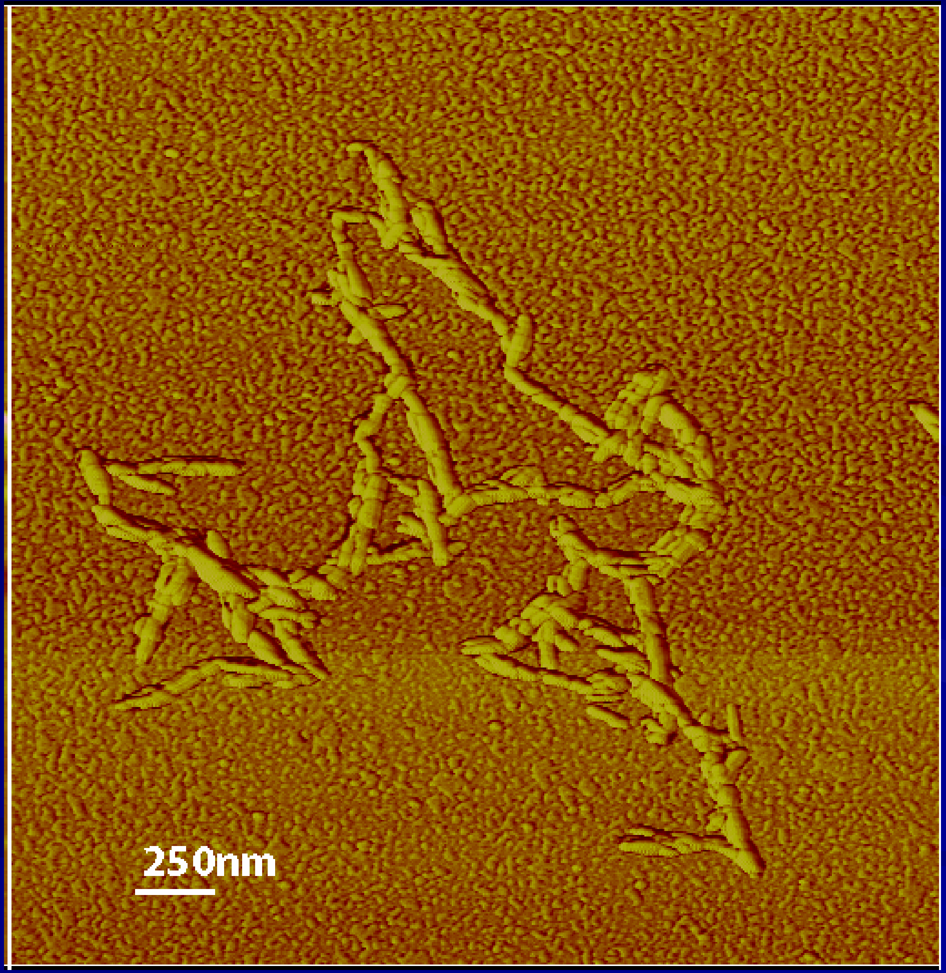
AFM image – ssDNA-CNXL



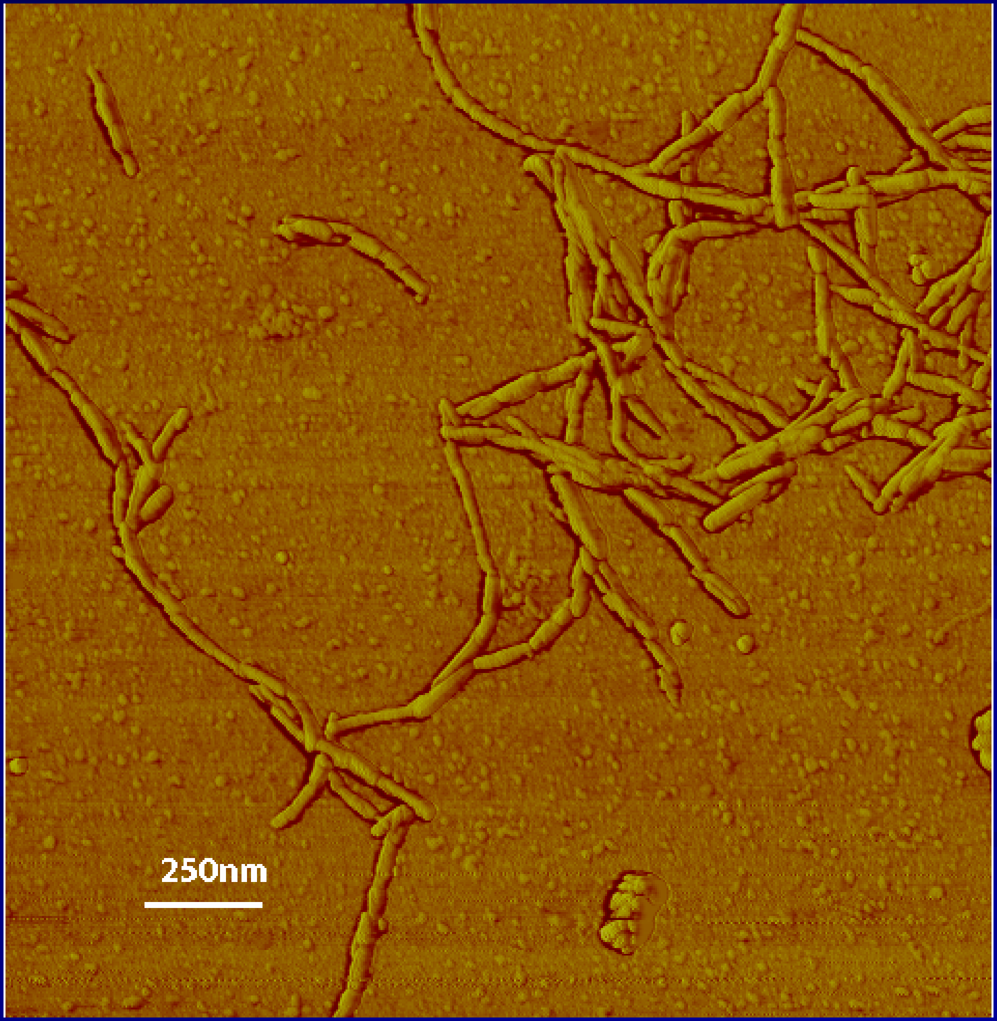
AFM image – duplex DNA-CNXL



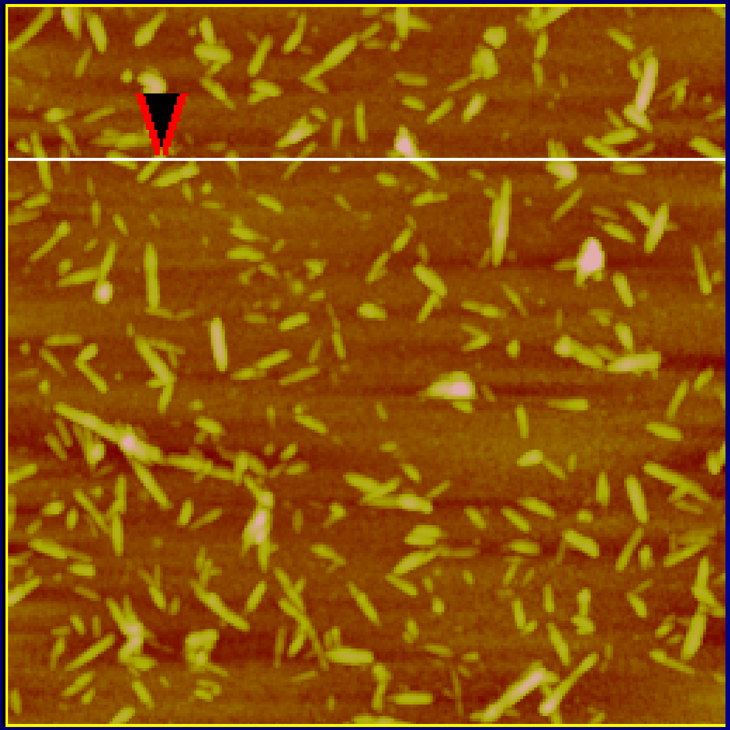
AFM image – duplex DNA-CNXL

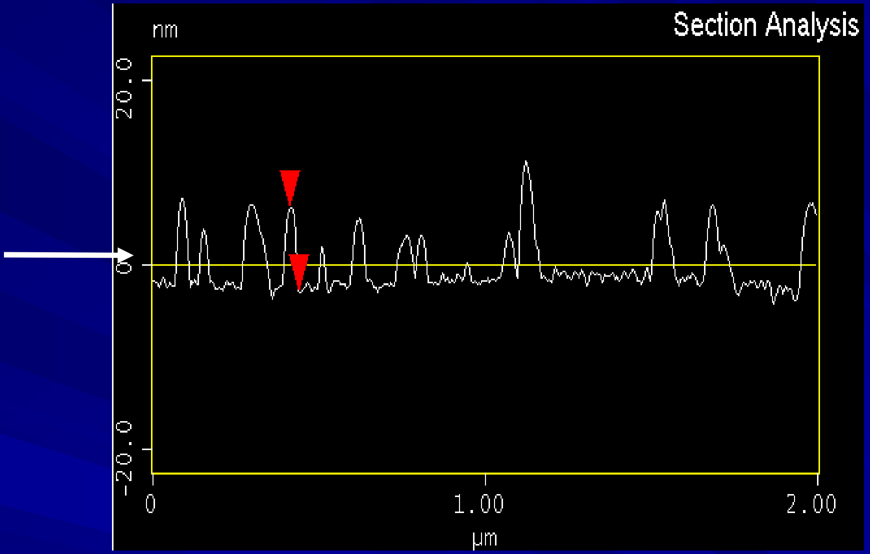


AFM image – duplex DNA-CNXL

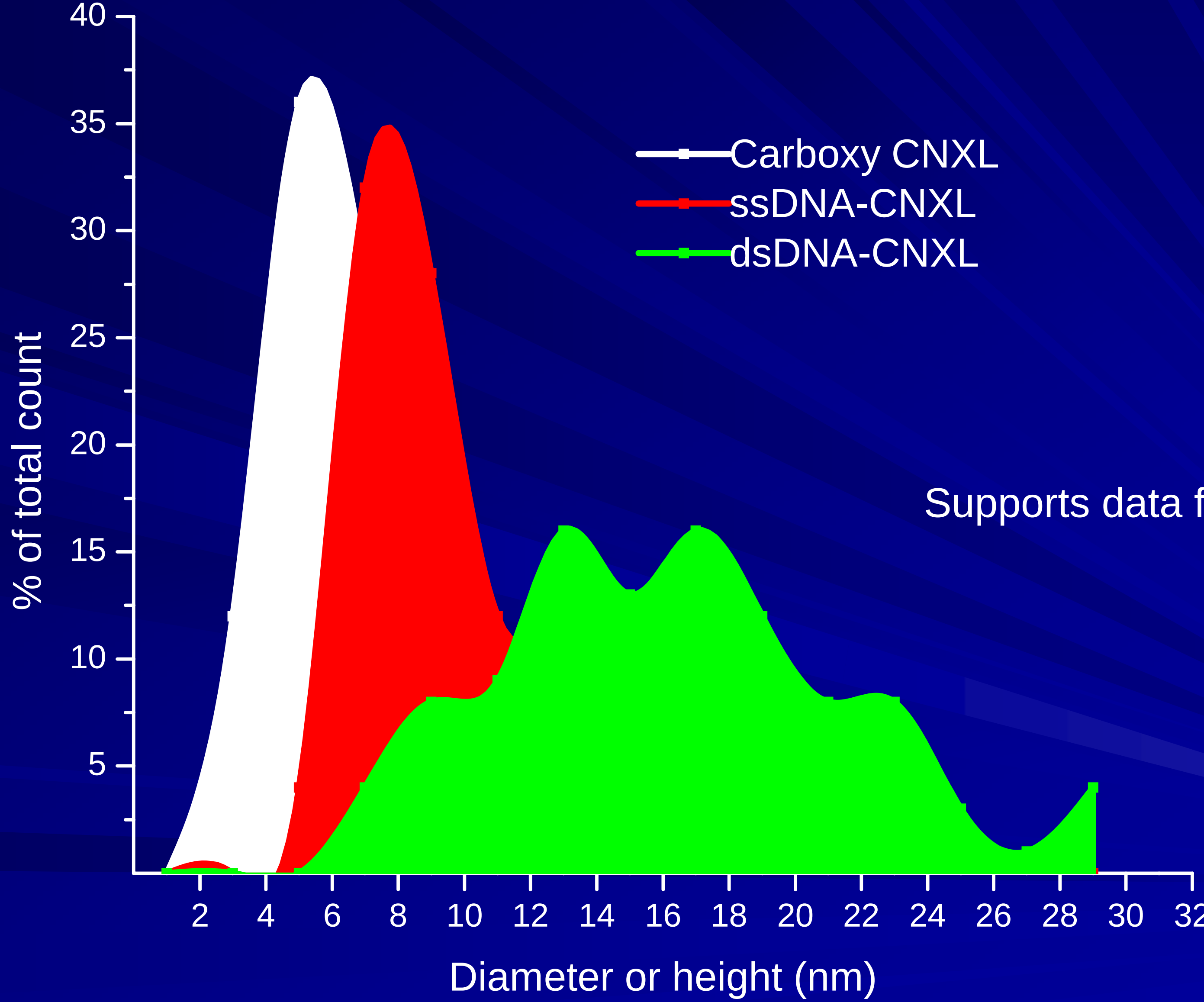


Section analysis of particle height





Particle height from section analysis



Supports data from DLS

Conclusions

- CNXLs were successfully carboxylated
- Carboxy-CNXLs were successfully grafted with DNA oligomers
- The DNA on the grafted CNXLs duplexed and bound the CNXLs together
- The duplex formation was reversible via raising the temperature above the DNA melting point
- While the goal of a new tissue engineering material remains distant, we believe we have shown that this concept has potential for use in thermoplastically formable implants with programmable in vivo dissolution and other bio-based nanomaterials