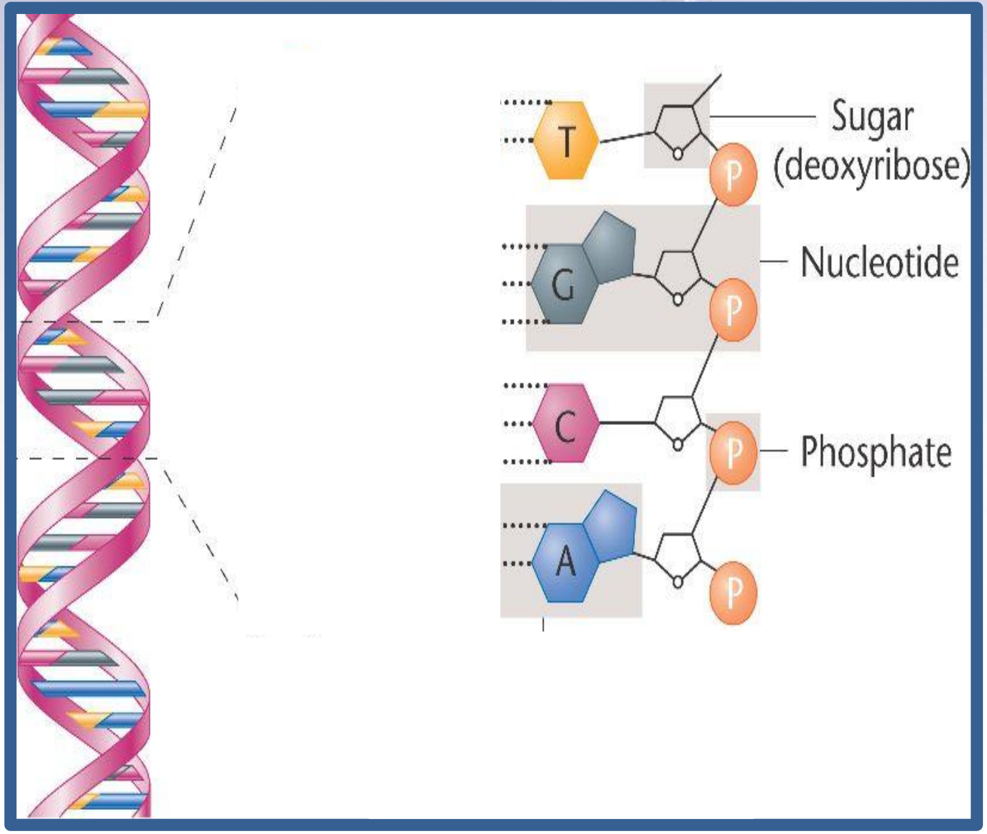
Spatial correlations in the melting transition of DNA

All you need is neutrons ILL seminars 27 October 2015

Introduction

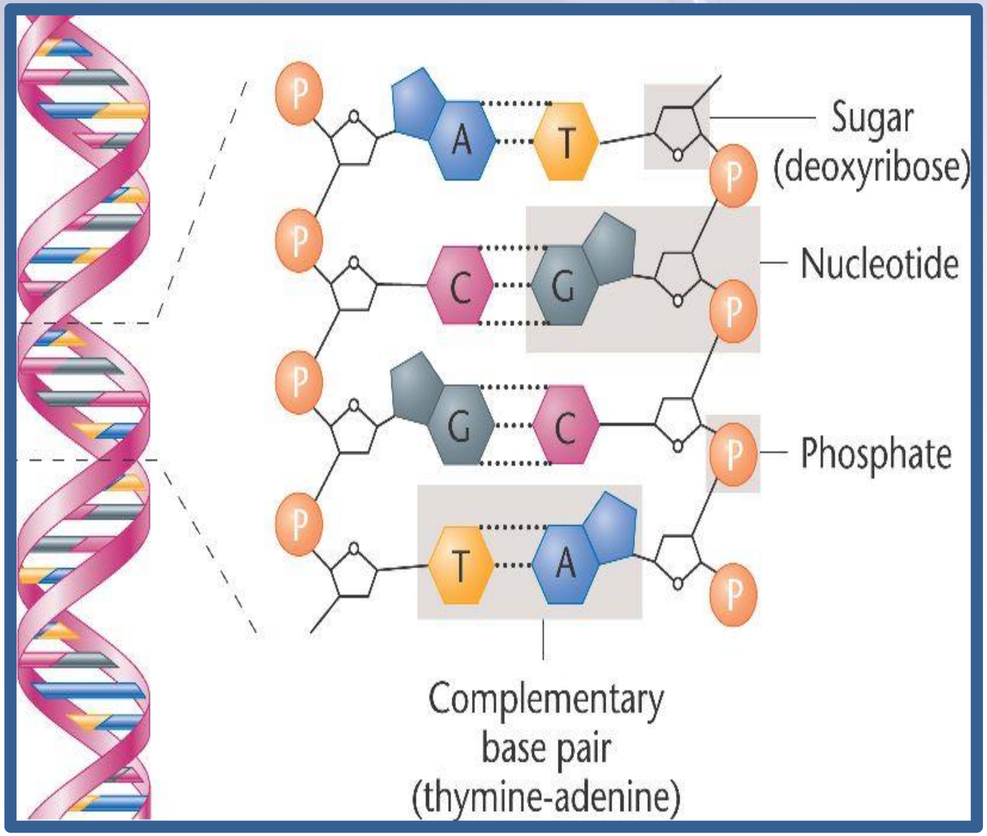
DNA STRUCTURE:



www.cliparthut.com

Introduction

DNA STRUCTURE:



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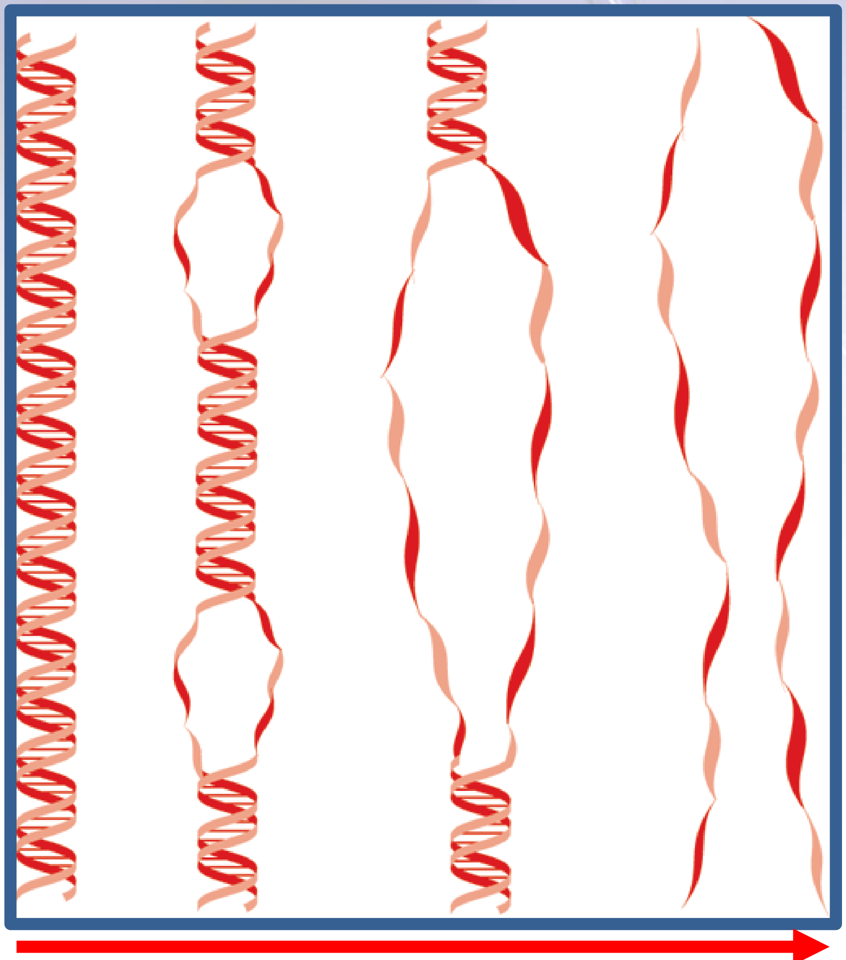
Introduction

DNA MELTING:



M. Peyrard, *Nature Physics* 2006, *2*, 13–14.

Introduction

Why melting transition?

Applications:
-Polymerase Chain reaction.
-High resolution melting analysis.

Theory:
- 1D phase transition.

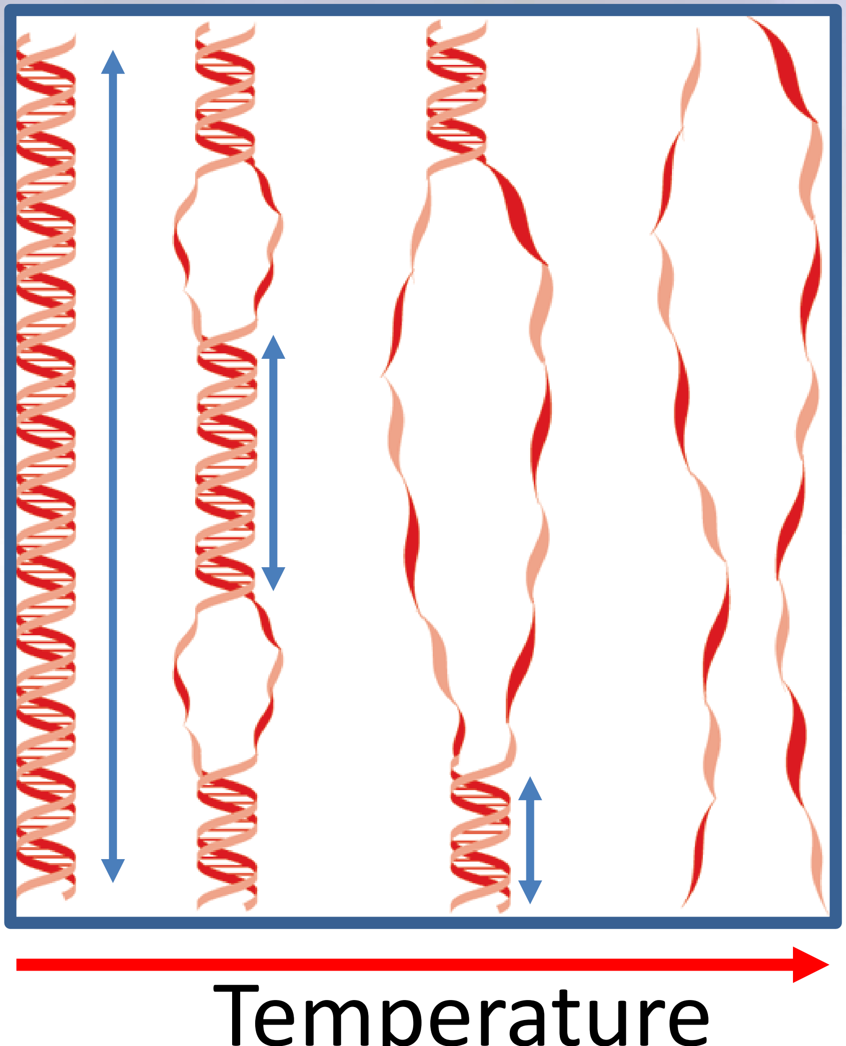
What´s original in our approach?

Previous techniques:
-DSC.
-Fluorescence.
-NMR.
-UV-Vis absorption.

Neutrons:
-Spatial correlation.

Introduction

CORRELATION LENGTH:



M. Peyrard, *Nature Physics* 2006, 2, 13–14.

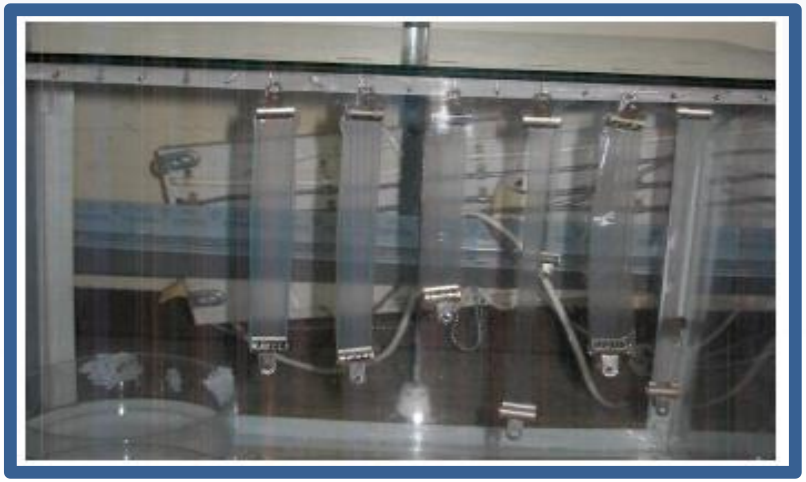
- The correlation length is related with the average size of the closed domains during the melting.
- Size of the open domains can be infer.

Methods

- Calorimetry, X-ray diffraction.
- Neutron diffraction:

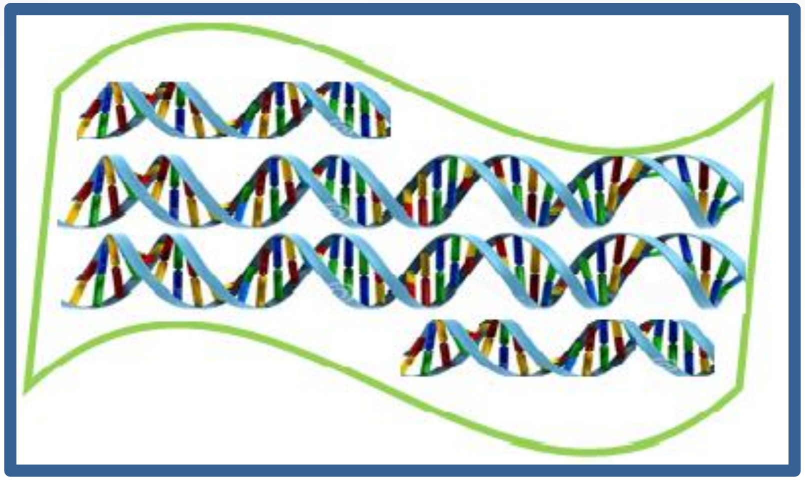
Fiber DNA → Semi-crystal → Bragg Peaks

DNA film samples:



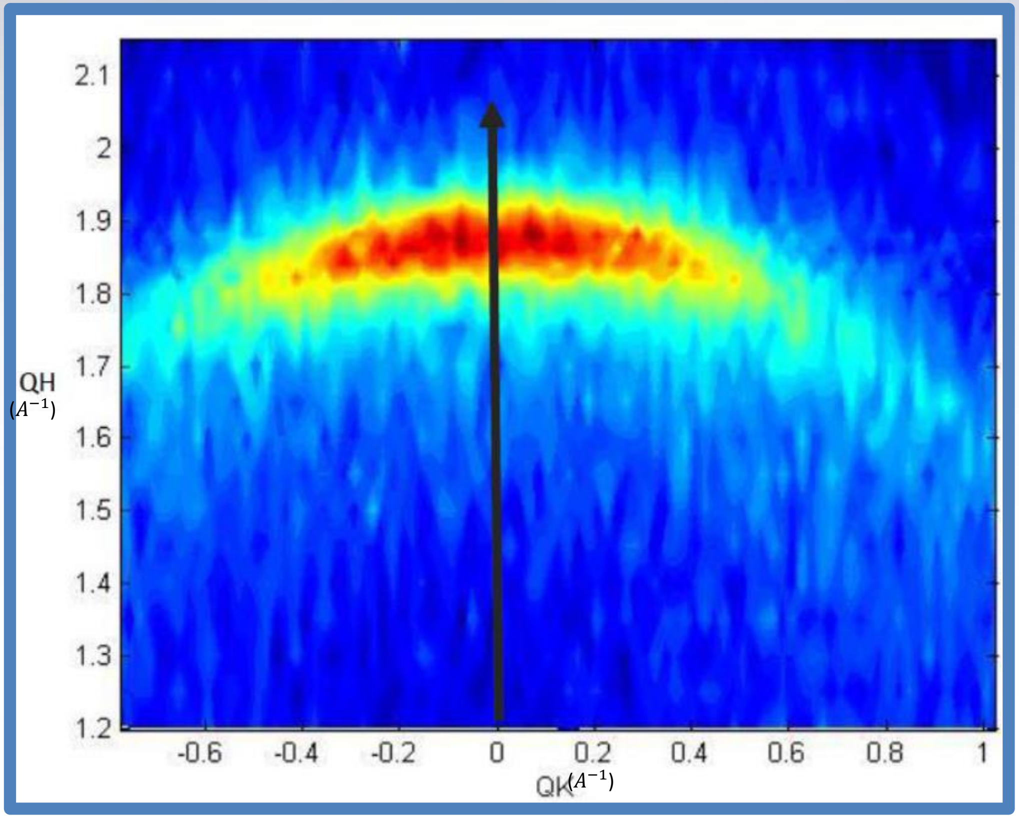
→

DNA-orientation in the film:



Methods

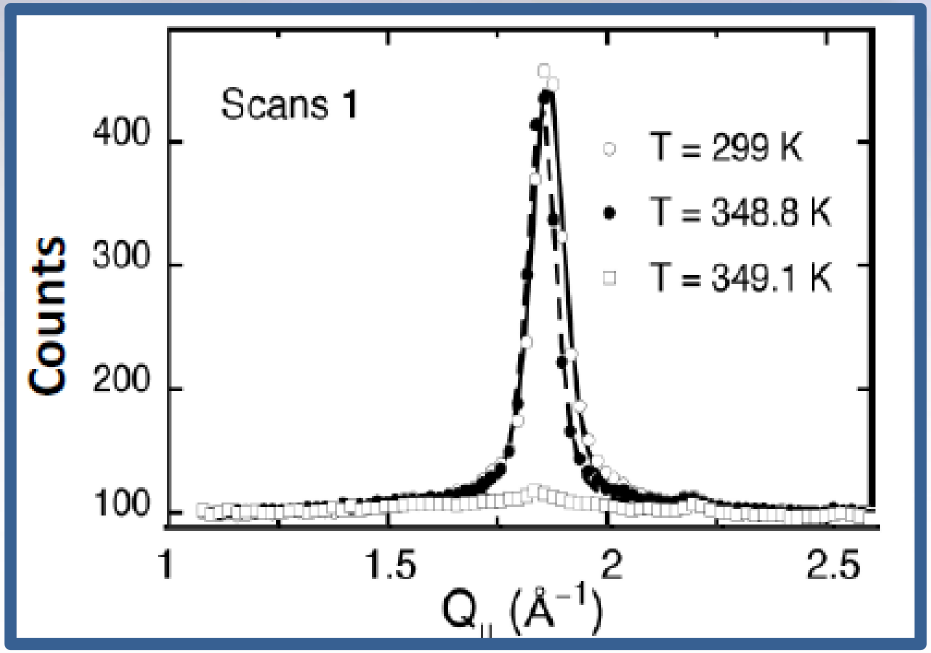
Fiber DNA → Semi-crystal → Bragg Peaks



- $Q_H = 1.85\ A^{-1}$ →**dspacing=3.4 A**
- Related with the correlation of two closed base-pairs.
- The scattering elements which are the origin of this peak are **closed base pairs**.

Dry Fiber

Longitudinal cut of the Bragg peak:

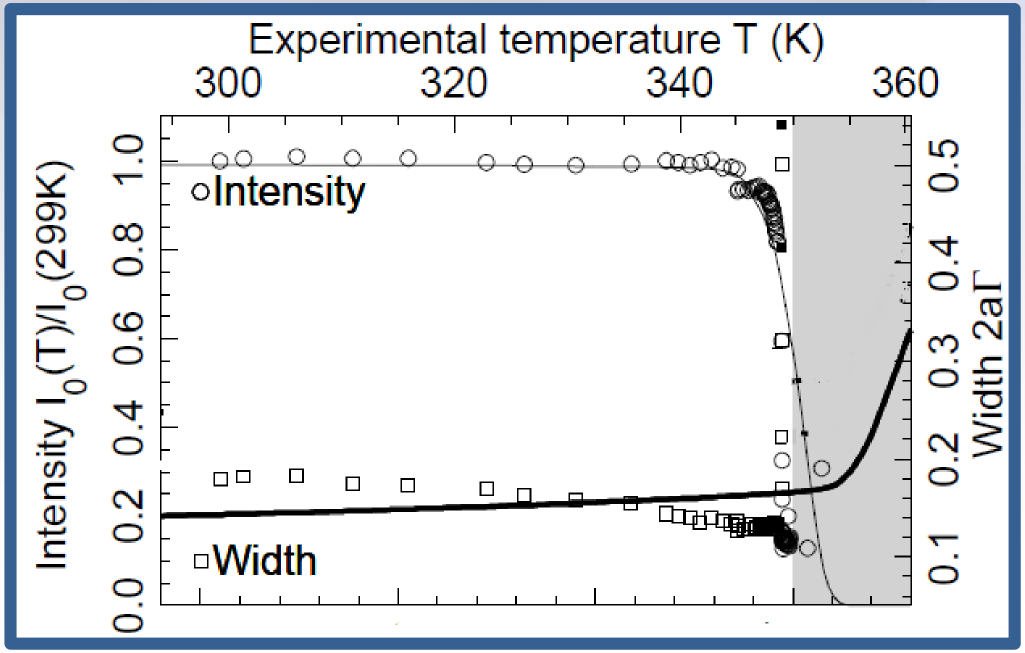


Wildes, A.; Phys. Rev. Lett. 2011, 106, 048101.

- I_0(integrated I) ∝ n° of closed base pairs.
- $\Gamma \propto \dfrac{1}{\text{correlation length}}$
- If T↑ thus I↓ and Γ↑

The Γ give us qualitative info about the evolution of the bubbles!

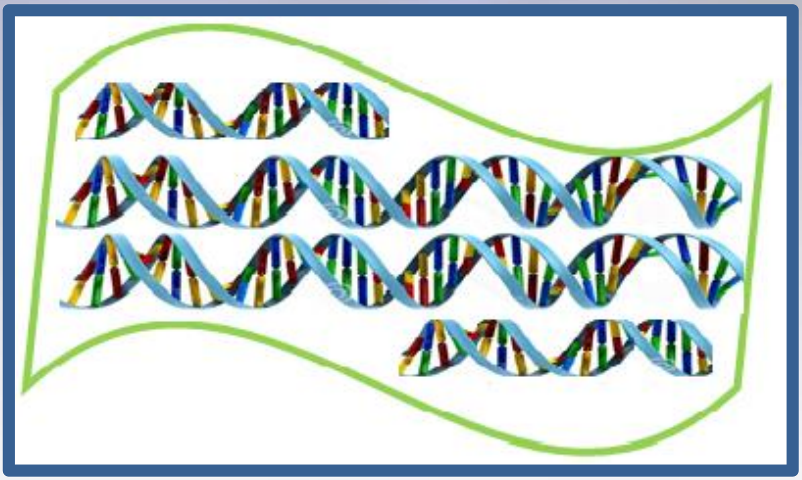
Dry Fiber

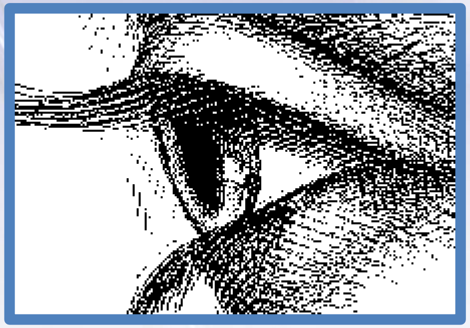


Wildes, A.; Phys. Rev. Lett. 2011, 106, 048101.

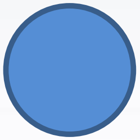
- Theory allows us to calculate the distribution of the open base pairs.
- Discrepancy of less than 15%.
- Problem: The model studies **independent molecules** of DNA.

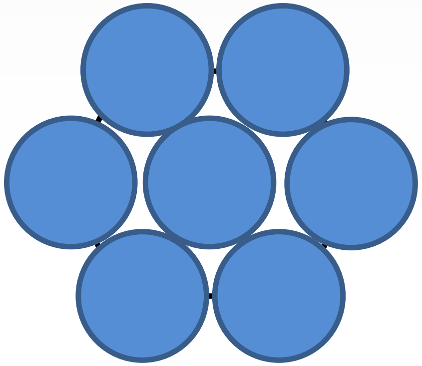
Osmotic pressure

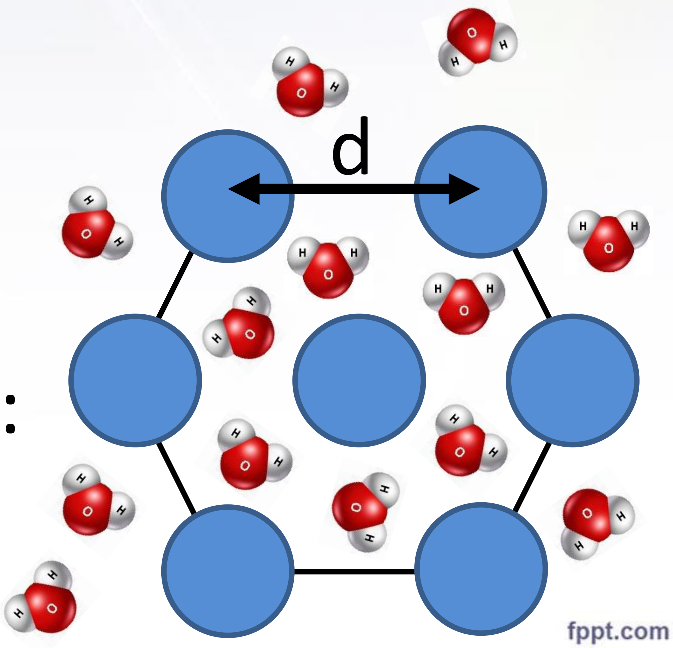




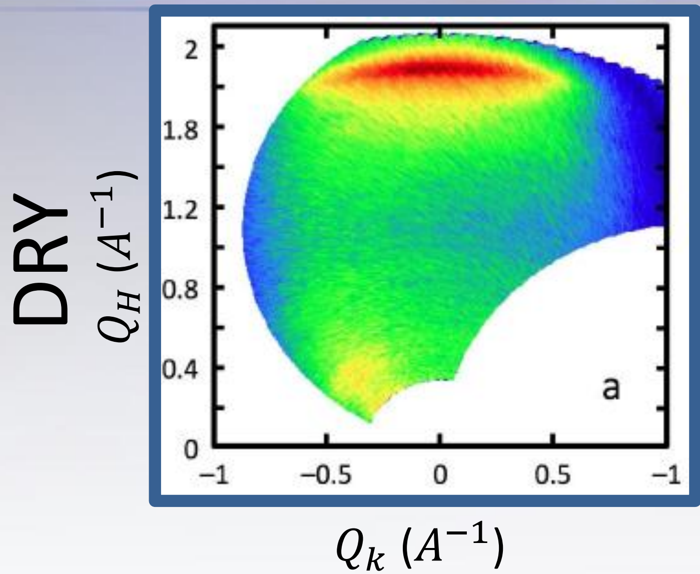
http://www.ralphmag.org

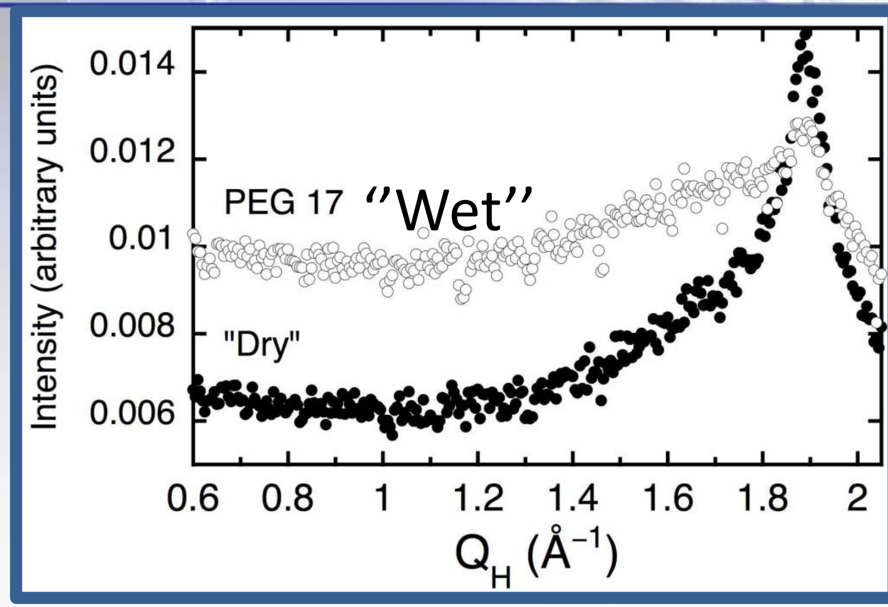
 =DNA molecule

DRY film: 

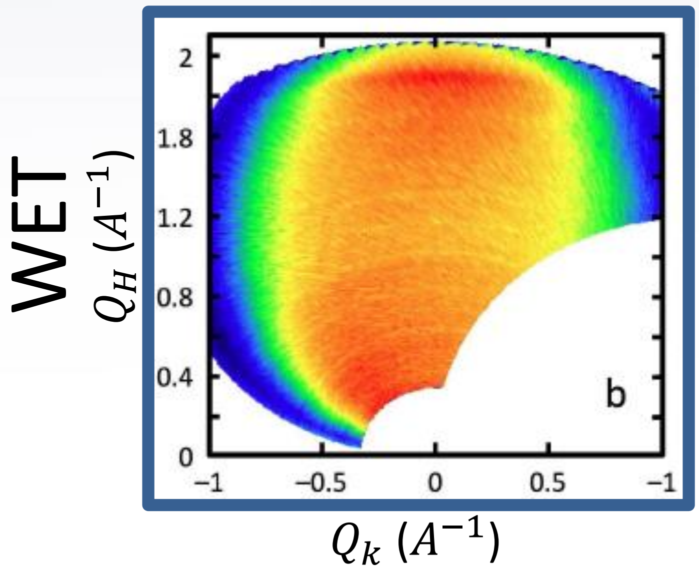
Wet fiber: 

Wet Fiber





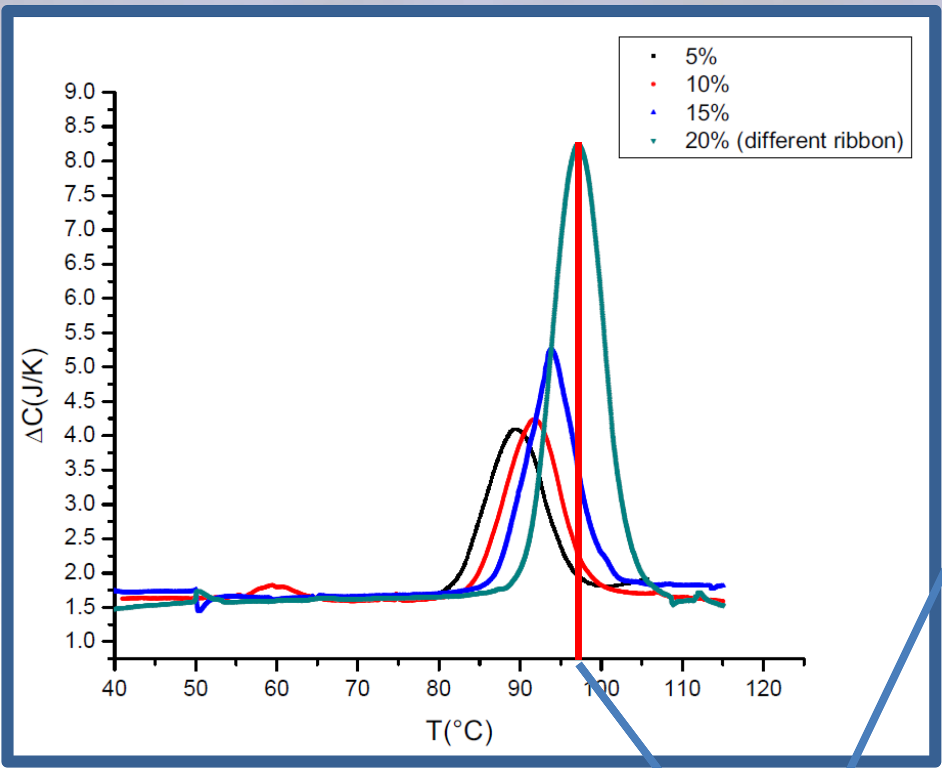
J. Phys. Chem. B, 2015, 119 (12), pp 4441–4449



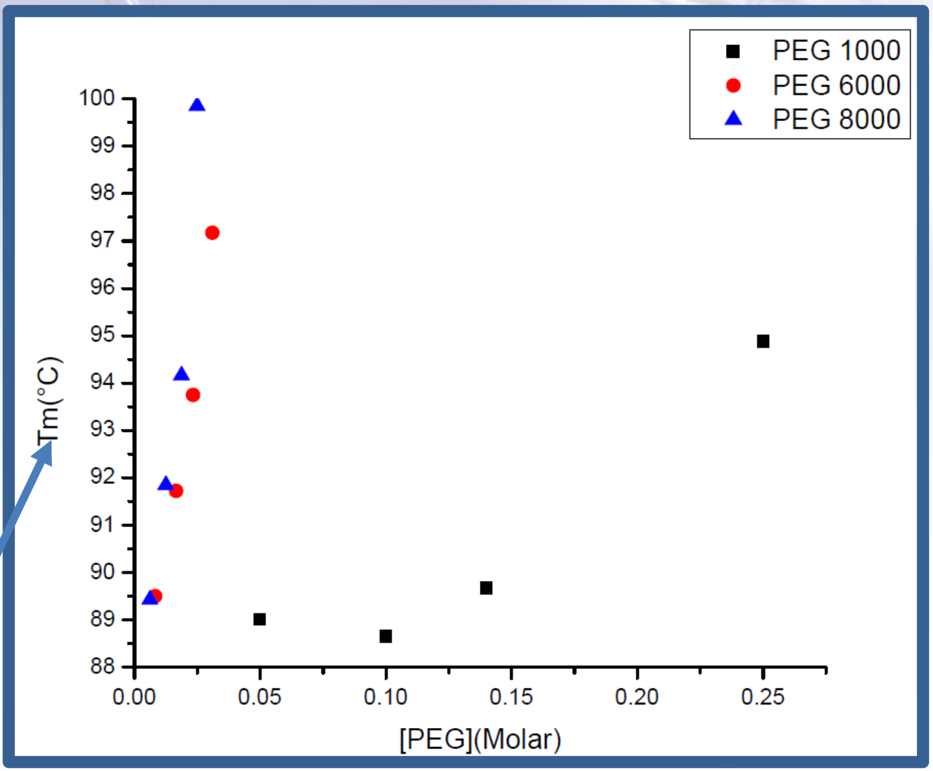
Problems
- High background.
- Peak anisotropy.
- Impossibility of a complete melting.

Calorimetry

Melting curves:



Tm vs. PEG concentration:

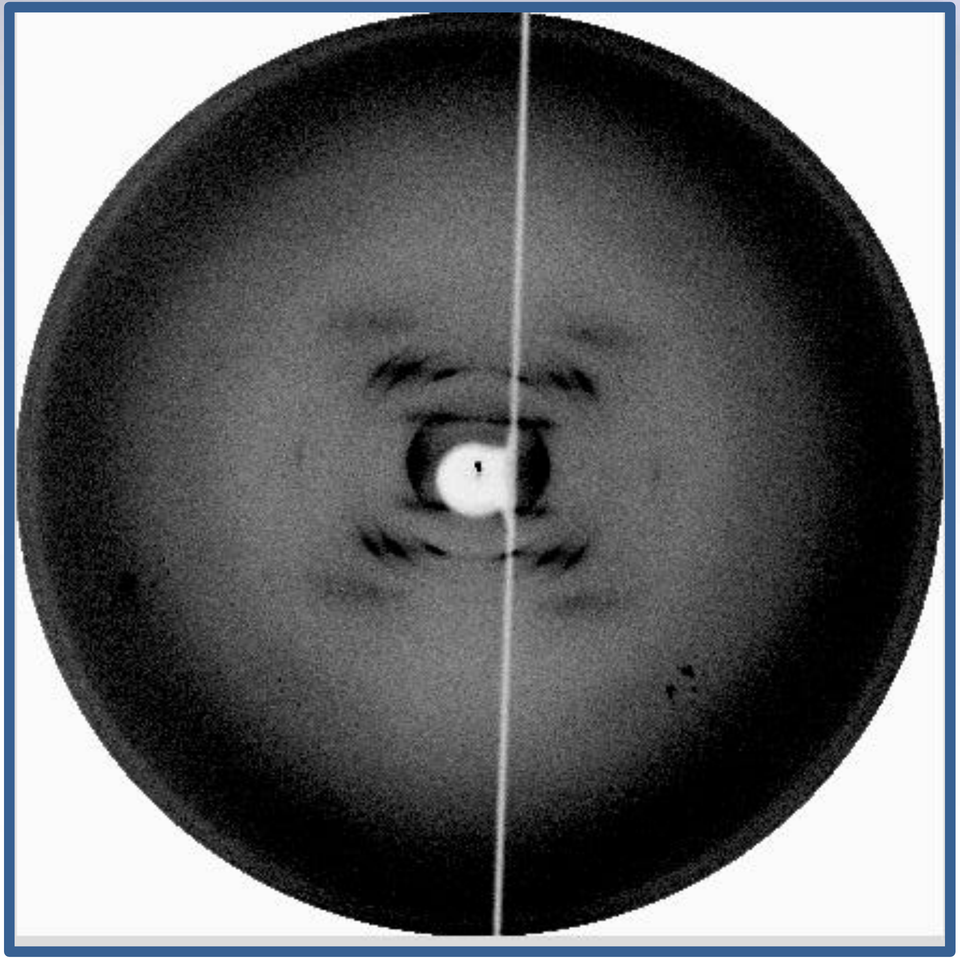


Melting temperature (T_m): Middle point of the transition.

Using a shorter PEG is better!

X-Ray diffraction

DNA in 66% Ethanol solution:

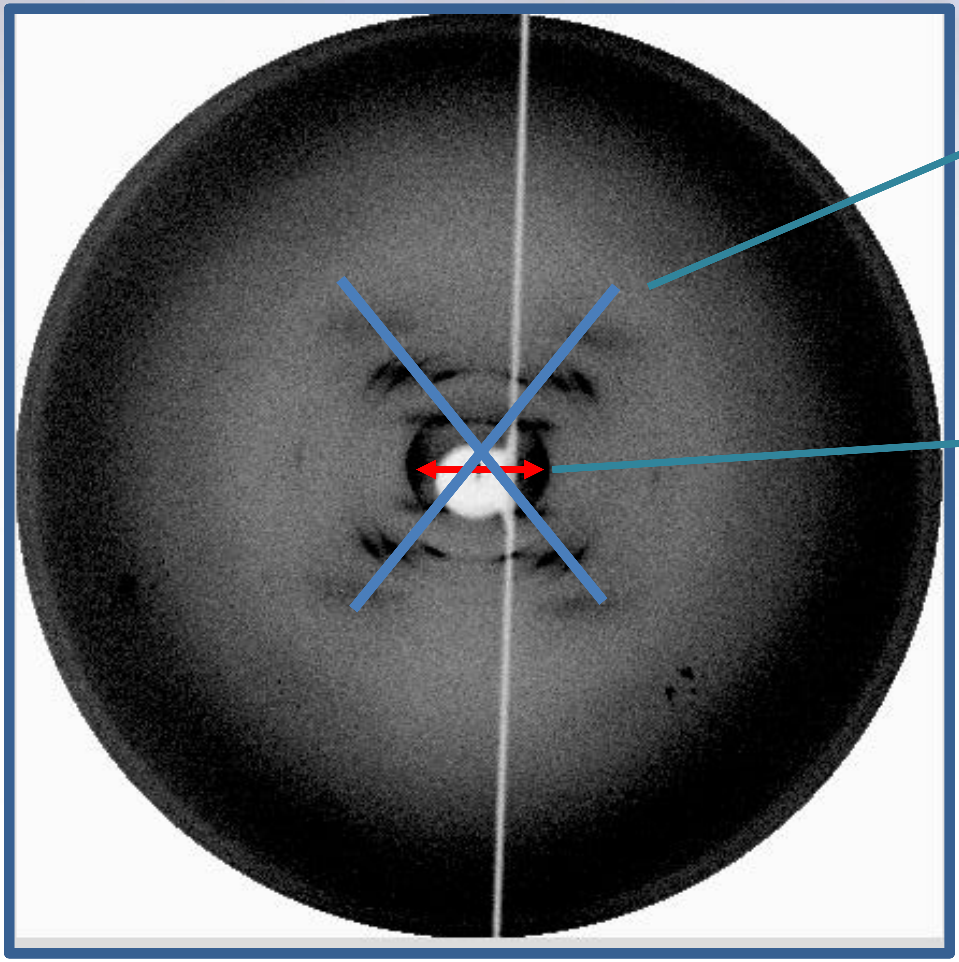


When we put the DNA in ethanol/water mixtures….

- …will it still be crystalline?
- …will the intermolecular space grow?

X-Ray diffraction

DNA in 66% Ethanol solution:



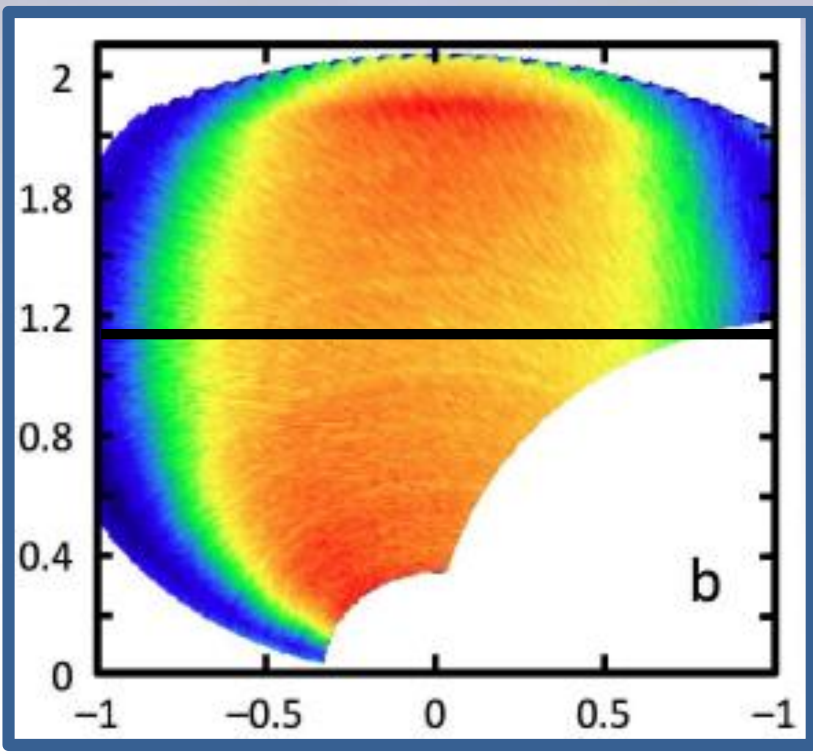
The X pattern is a sign of the semicrystalline B form.

Equatorial spacing $\propto \frac{1}{d}$

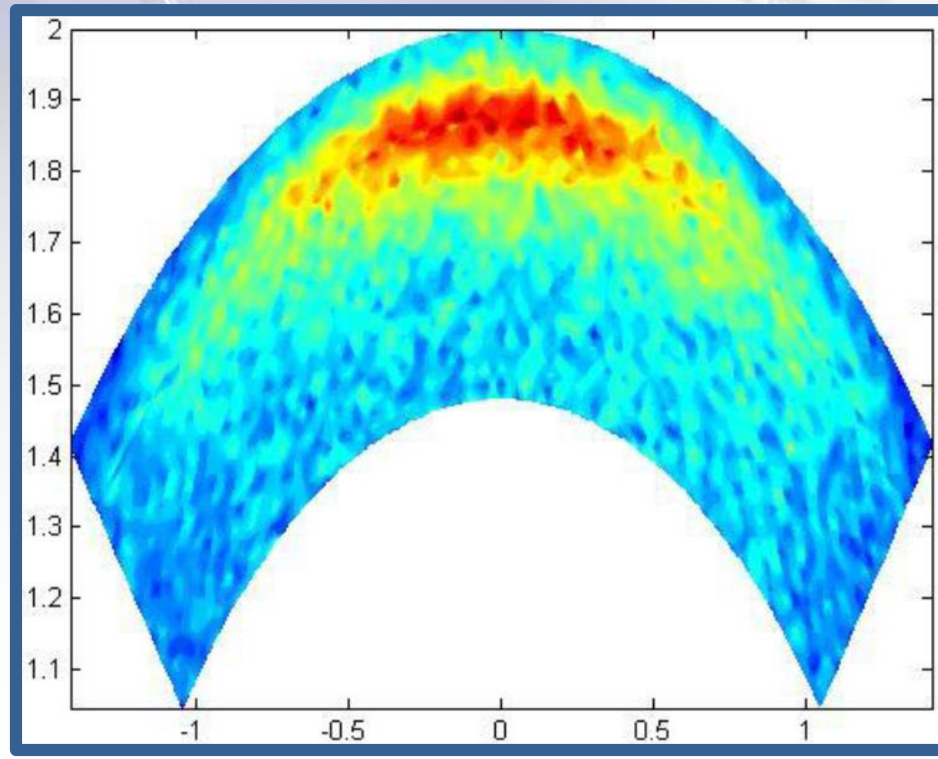
d=intermolecular spacing

Wet Fiber 2

Before



After



$Q_H\ (A^{-1})$

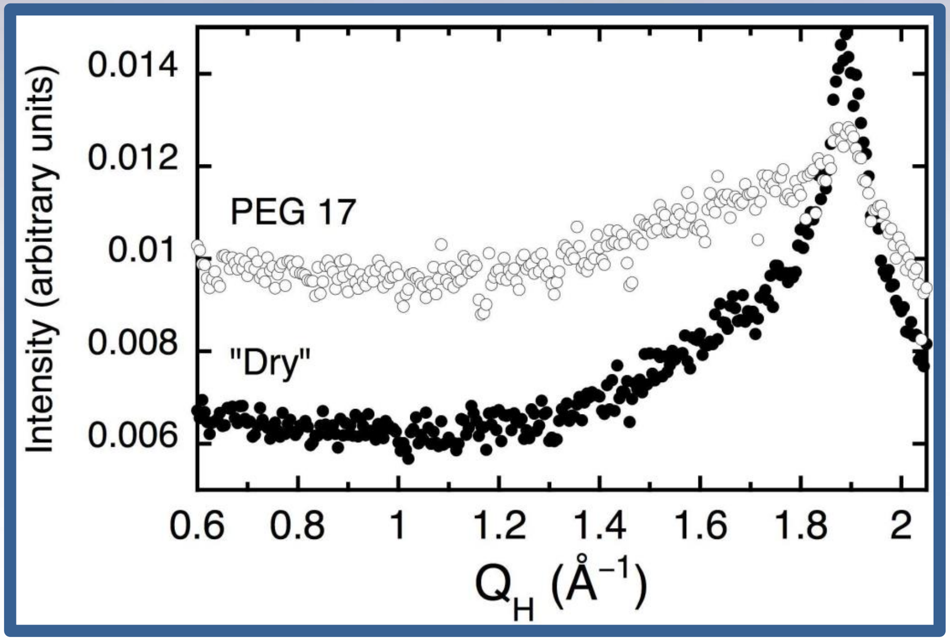
J. Phys. Chem. B, 2015, 119 (12), pp 4441–4449

$Q_k\ (A^{-1})$

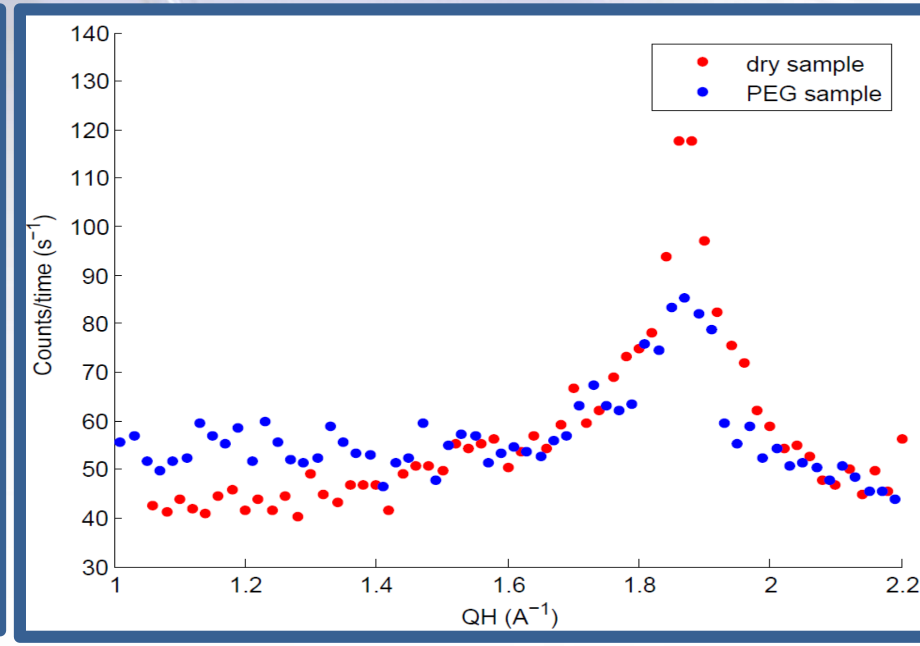
Better ratio peak-signal to noise!

Wet Fiber 2

Before



After



More symmetric peak!

Conclusions

- The melting transition in DNA is not fully understood due to the lack of spatial information.

- Neutrons can account for this lack.

- Osmotic pressure methods allow us to study the melting of fiber DNA in solution and prove the effect of the confinement.

Acknowledgment

Thank you for your attention

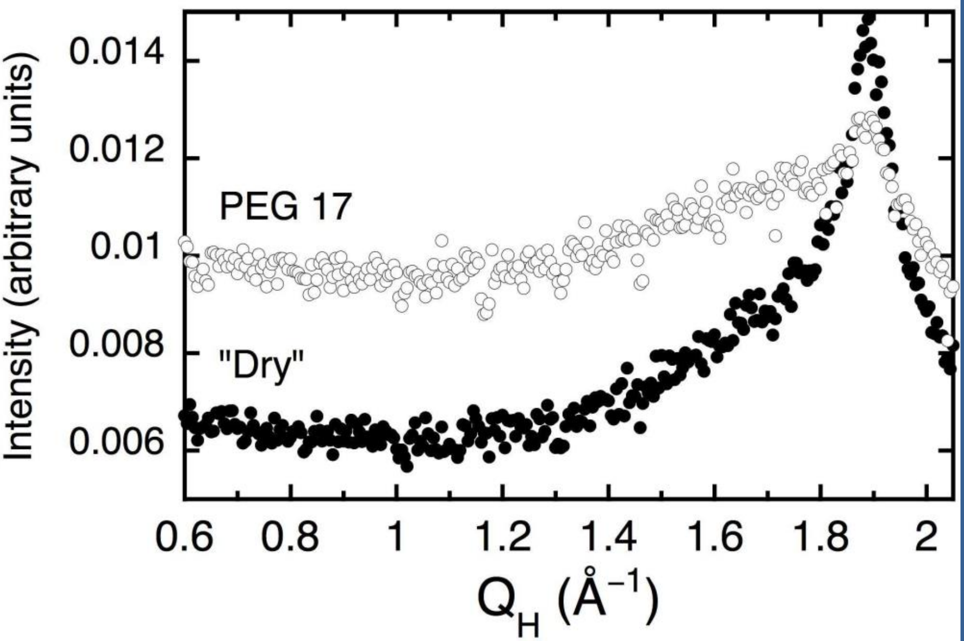
Andrew Wildes, Santiago Cuesta López, Michel Peyrard, Jean-Luc Garden, Gael Moiroux, David Hess, Matthew Blakeley, Diane Lançon, Hassan Belrhari, Eva María Villar Alvarez, Andrew MC Carthy…

NEUTRONS FOR SCIENCE

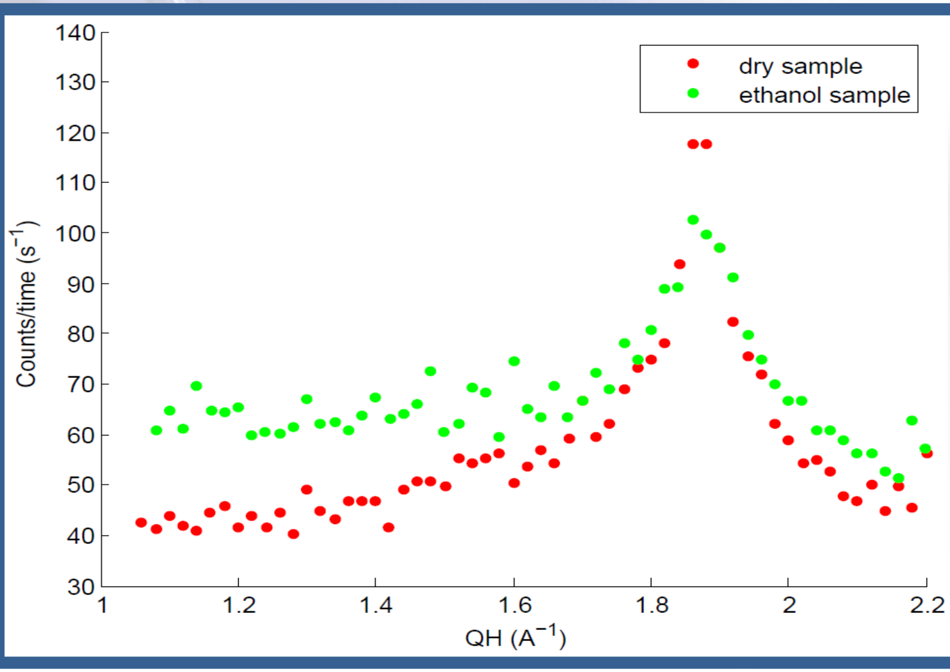
UNIVERSIDAD DE BURGOS

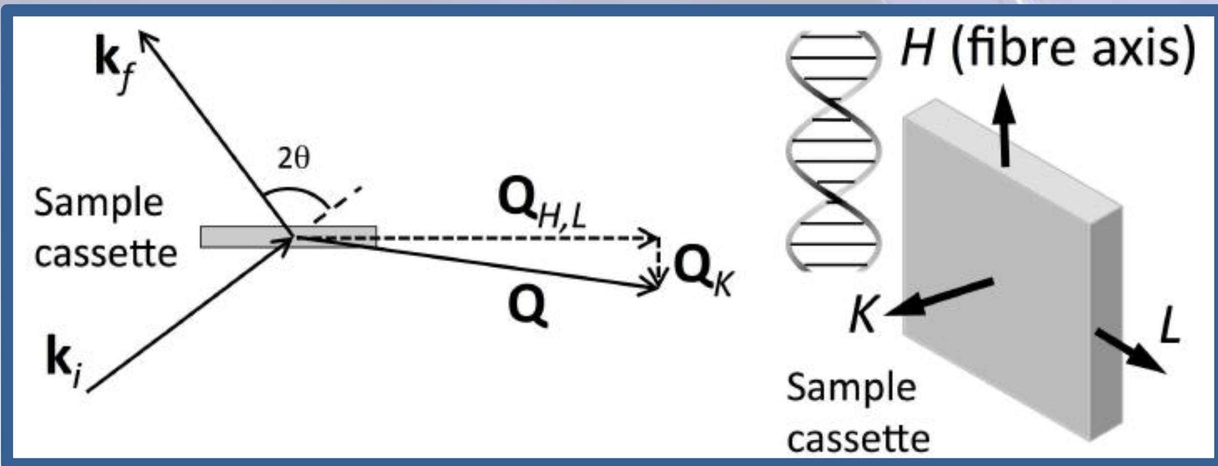
Wet Fiber

Before

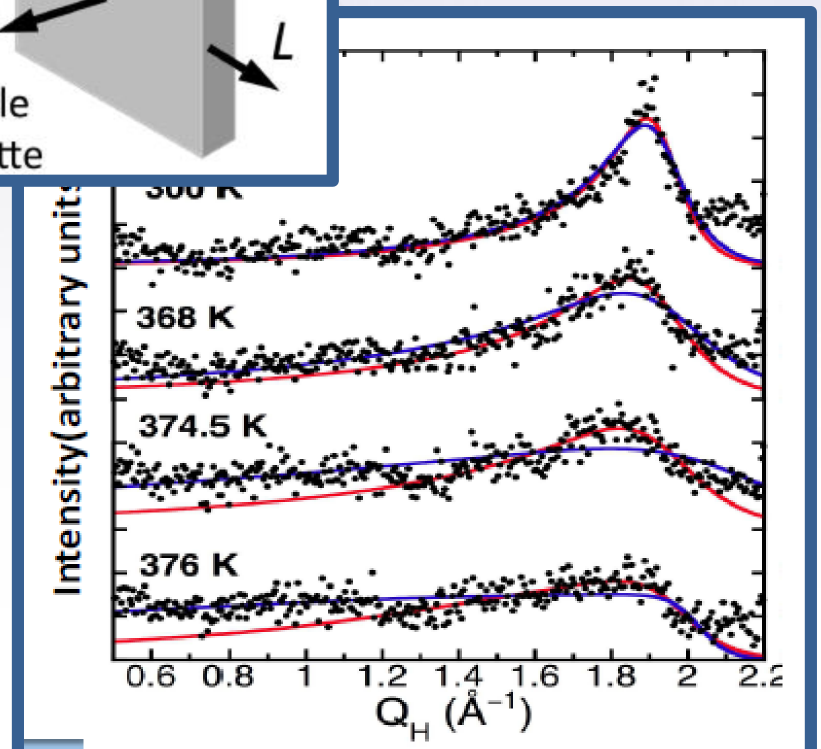


After

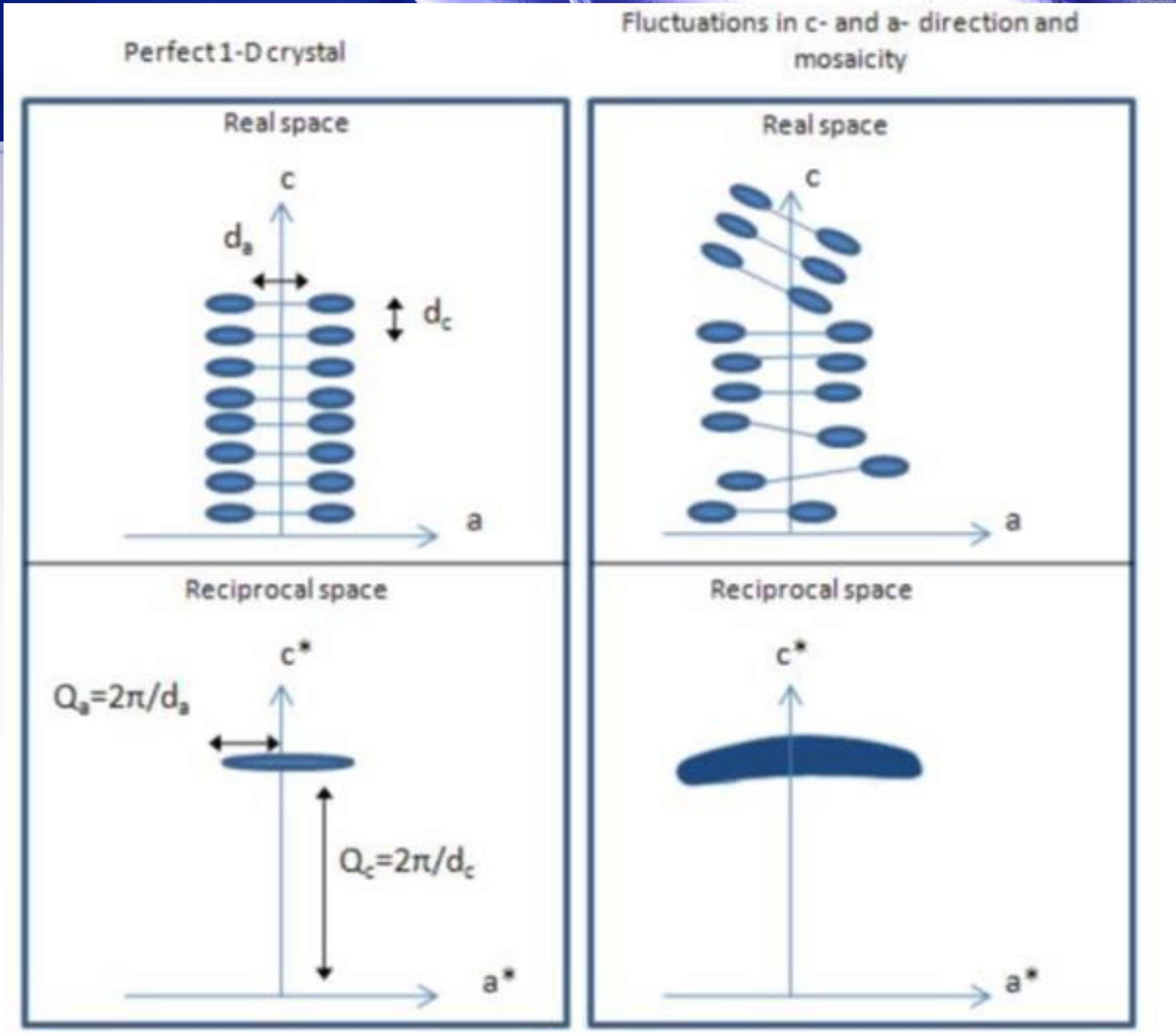




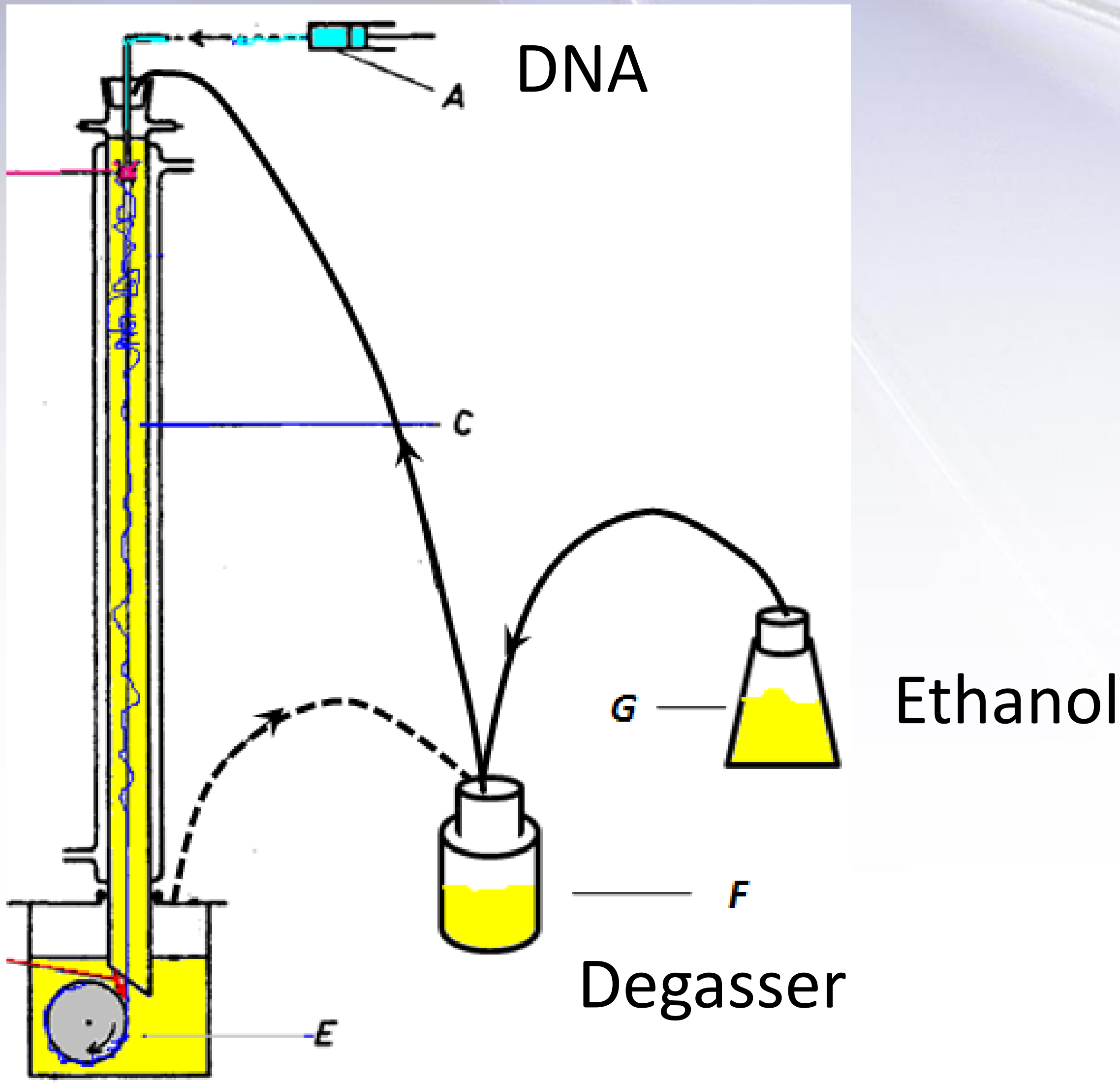
k_f
2θ
Sample cassette
$Q_{H,L}$
Q_K
Q
k_i
H (fibre axis)
K
L
Sample cassette



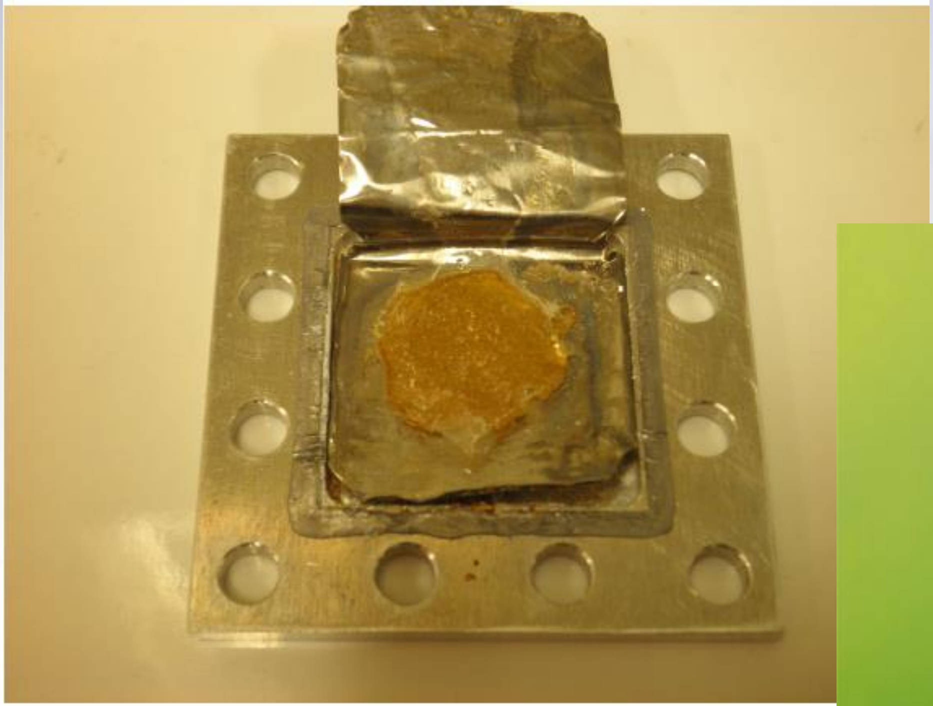
368 K
374.5 K
376 K
0.6 0.8 1 1.2 1.4 1.6 1.8 2
Q_H ($Å^{-1}$)



Perfect 1-D crystal
Fluctuations in c- and a- direction and mosaicity
Real space
c
d_a
d_c
a
Real space
c
a
Reciprocal space
c*
Q_a=2π/d_a
Q_c=2π/d_c
a*
Reciprocal space
c*
a*


A
DNA
C
G
Ethanol
F
E
Degasser

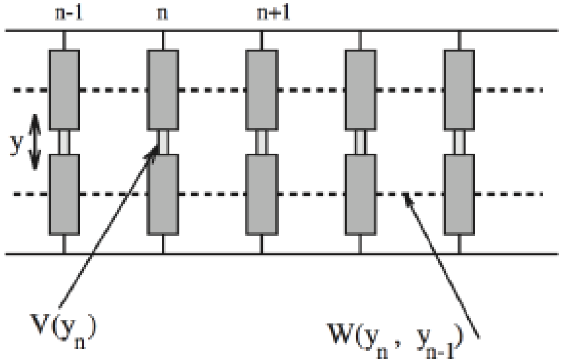
Neutrons

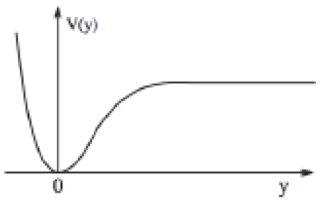


X rays

M. Peyrard, Nonlinearity **17** (2004) R1

$$H_y = \sum_{j=1}^{N-1} W\left(y_j, y_{j+1}\right) + \sum_{j=1}^{N} V_j\left(y_j\right)$$





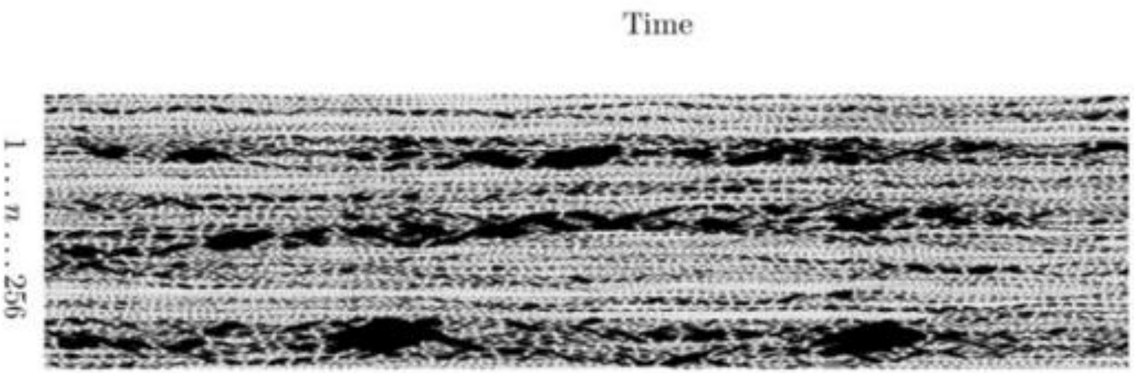
$$W\left(y_j, y_{j+1}\right) = \frac{1}{2}k\left[1 + \rho e^{-b\left(y_j + y_{j+1}\right)}\right]\left(y_j - y_{j+1}\right)^2$$

$$V_j = D_j\left(1 - e^{-\alpha_j y_j}\right)^2$$

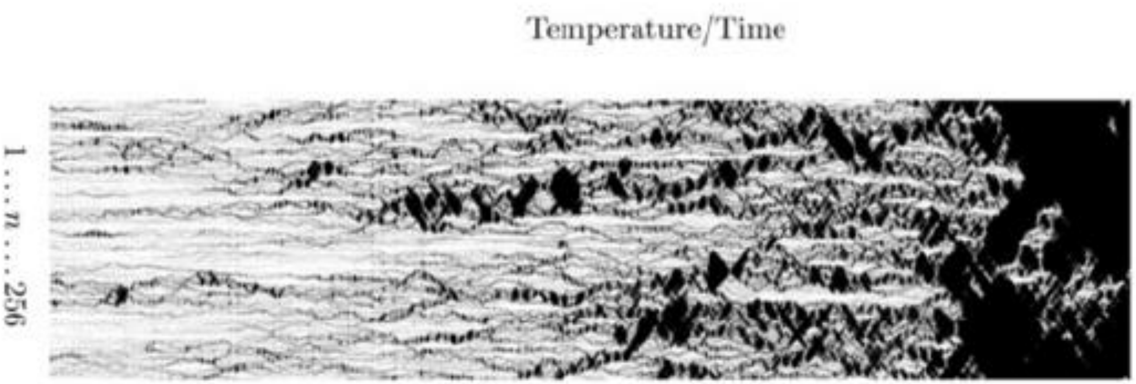
7 parameters:

k = speed of sound
ρ = relative strength of stacking
b = inverse range of stacking
$D_{AT,\,GC}$, $\alpha_{AT,\,GC}$ = Morse potentials

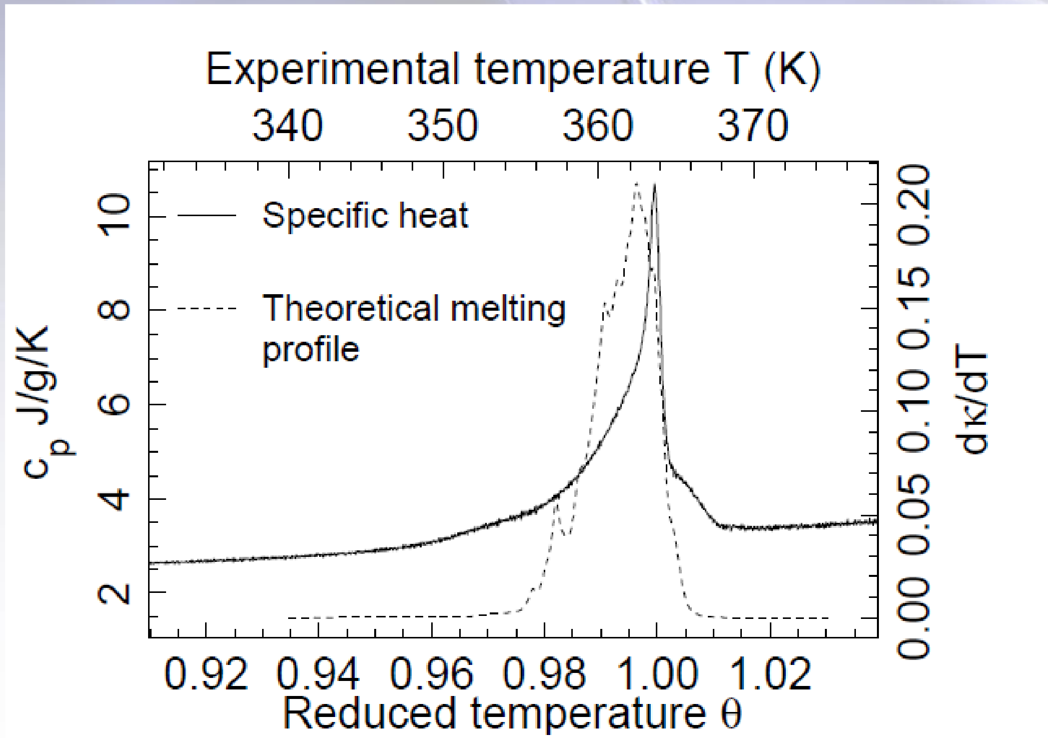
Simulations in M. Peyrard, Nonlinearity **17** (2004) R1

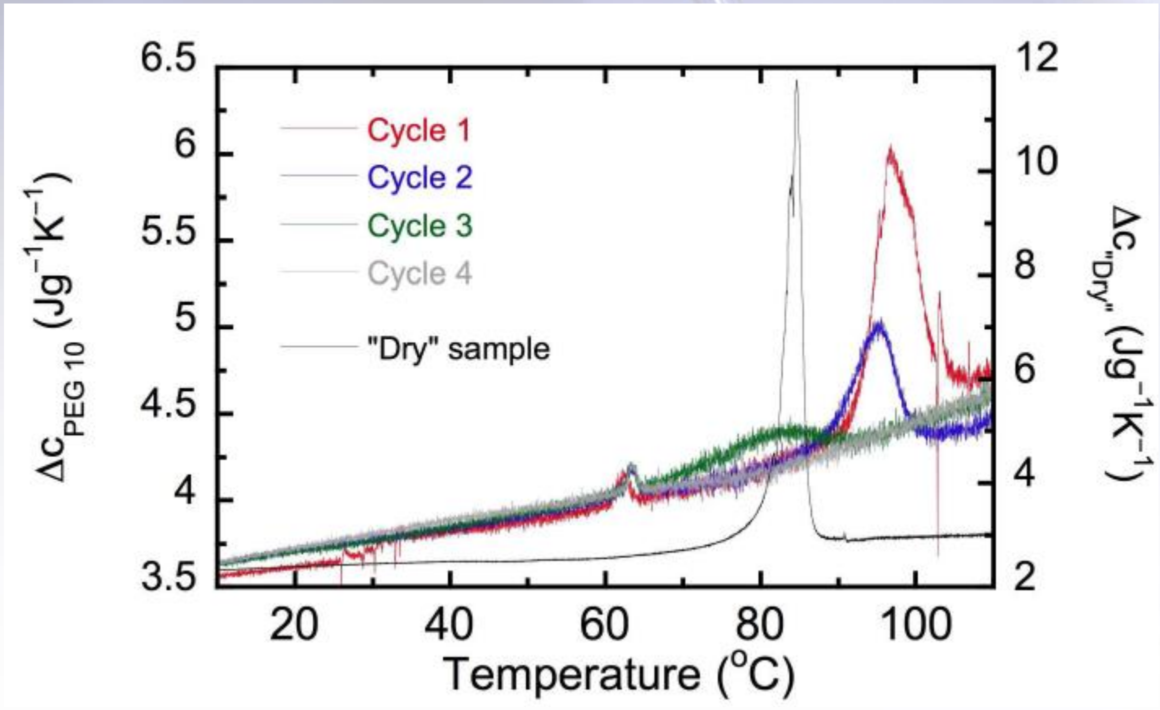


T fixed at 340K

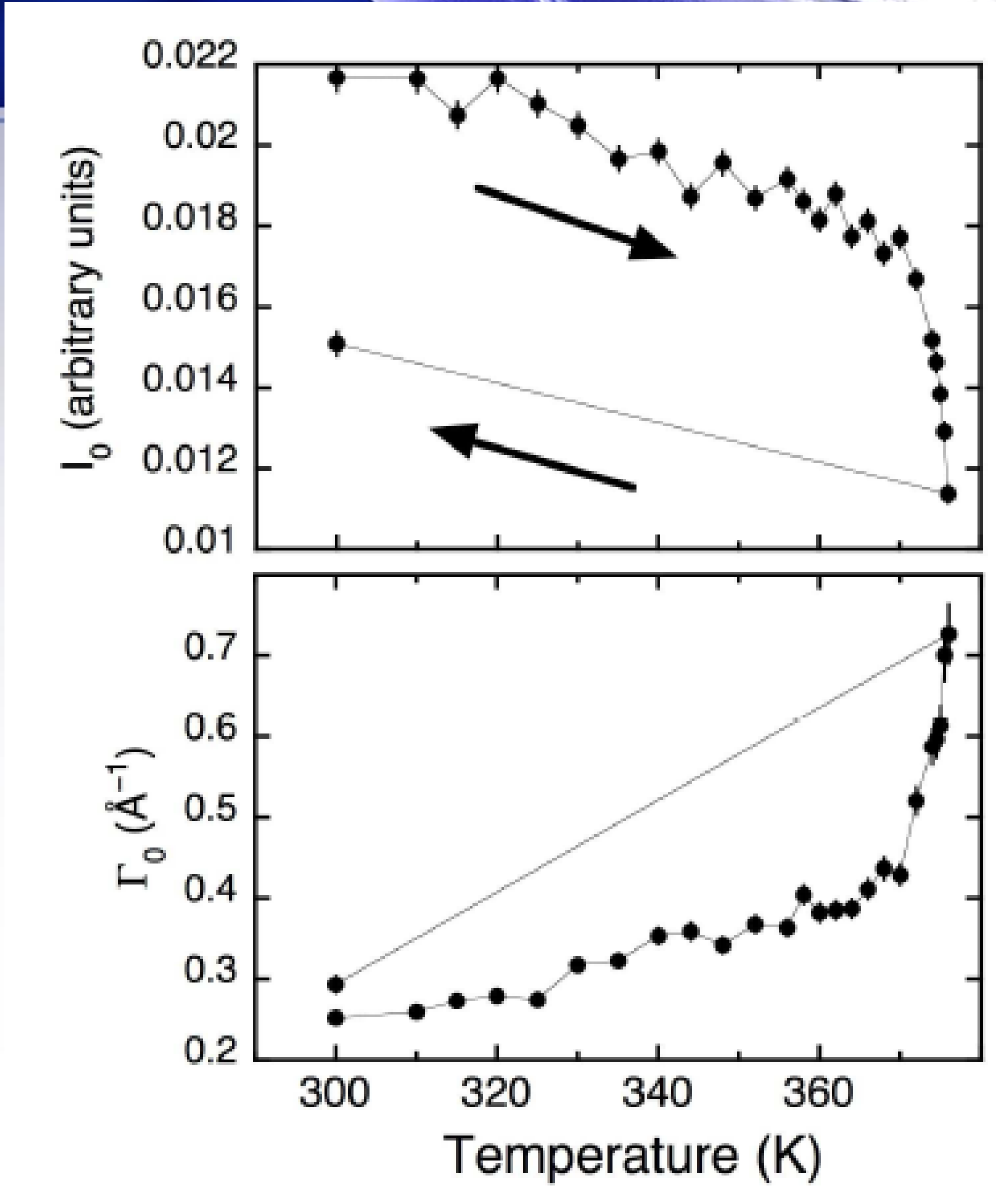


T increasing with time

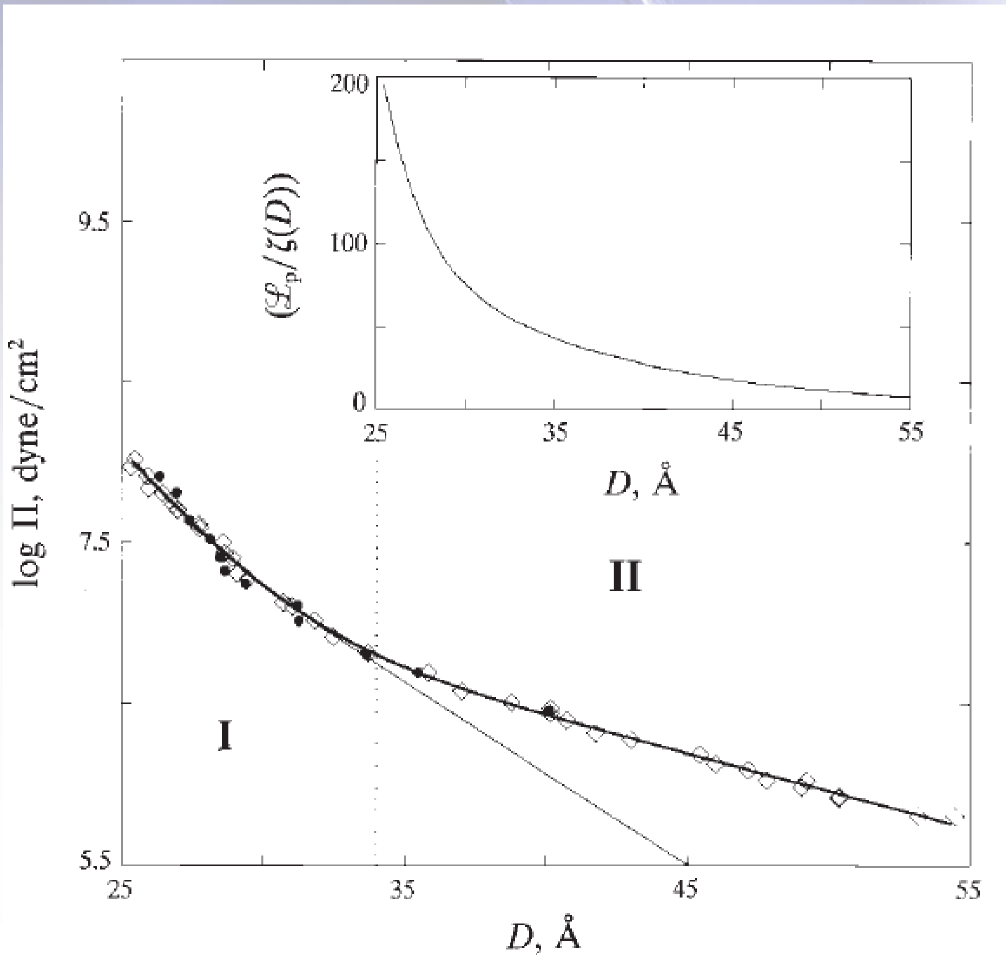

Experimental temperature T (K)
340
350
360
370
Specific heat
Theoretical melting profile
c_p J/g/K
dκ/dT
2
4
6
8
10
0.00 0.05 0.10 0.15 0.20
0.92
0.94
0.96
0.98
1.00
1.02
Reduced temperature θ

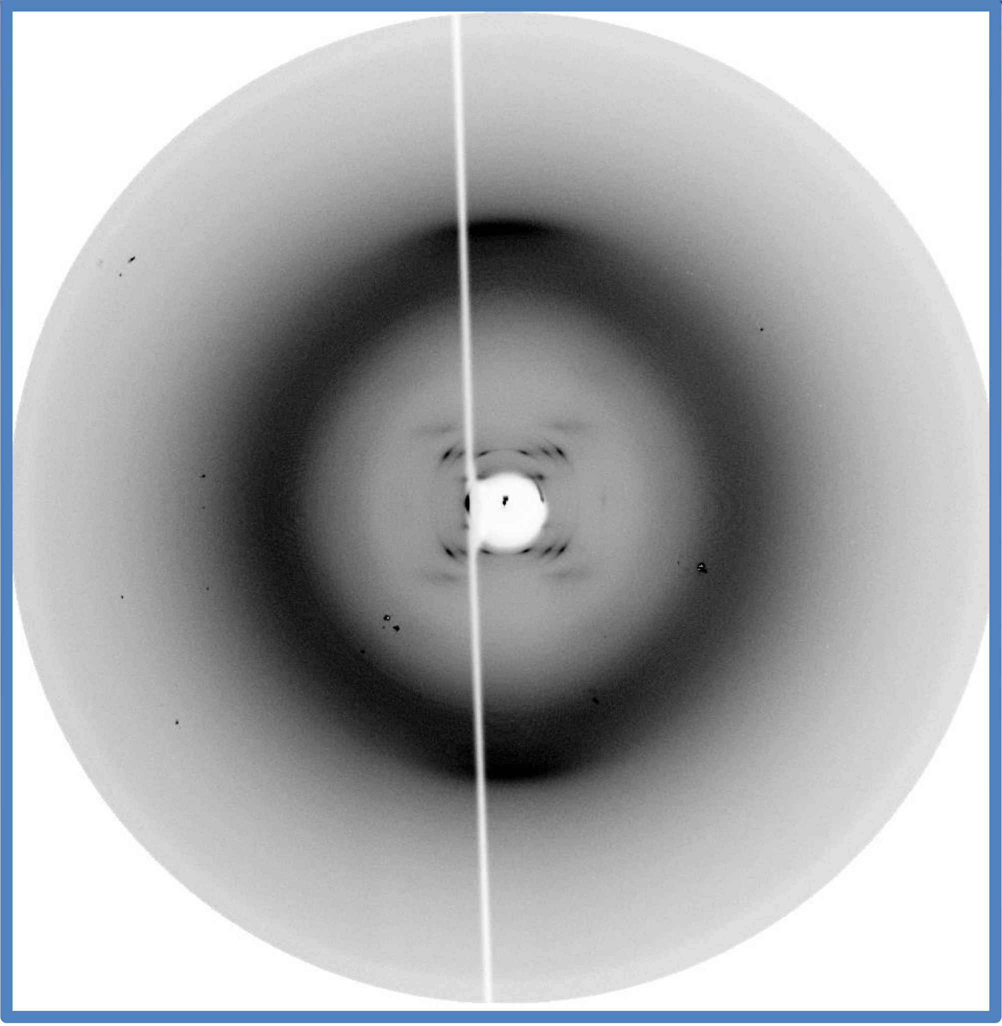


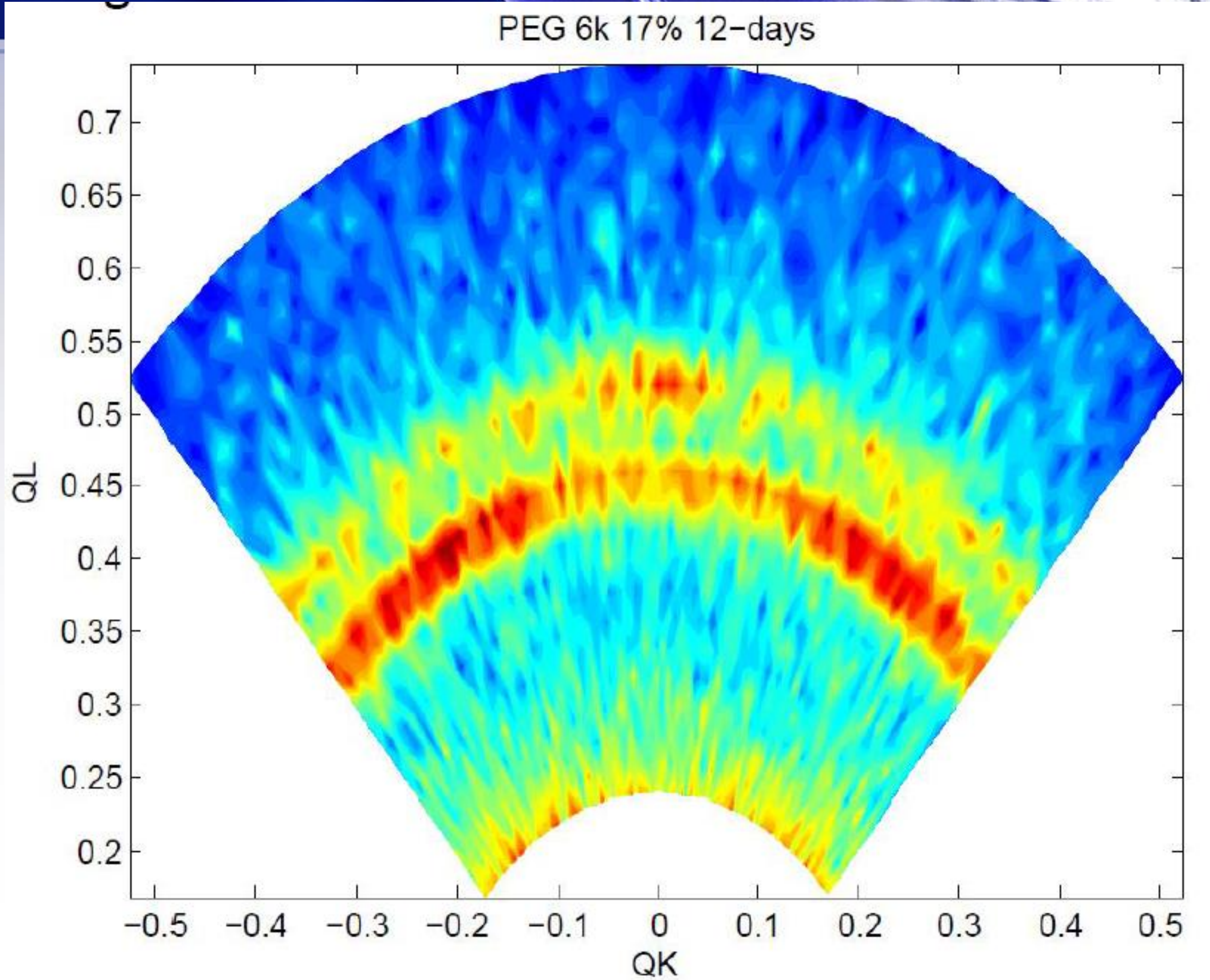
6.5
6
5.5
5
4.5
4
3.5
12
10
8
6
4
2
Cycle 1
Cycle 2
Cycle 3
Cycle 4
"Dry" sample
$\Delta c_{PEG\ 10}$ ($Jg^{-1}K^{-1}$)
$\Delta c_{"Dry"}$ ($Jg^{-1}K^{-1}$)
20
40
60
80
100
Temperature (^{o}C)

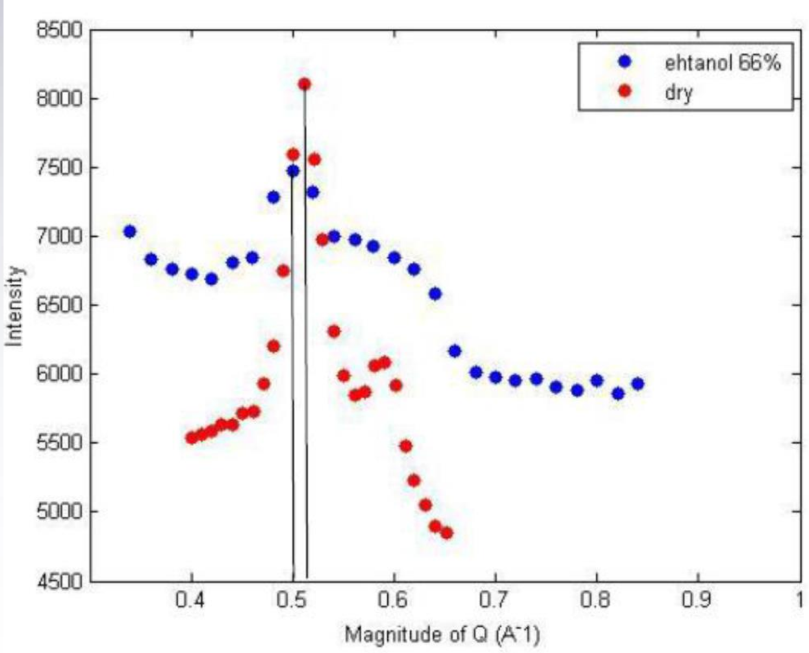


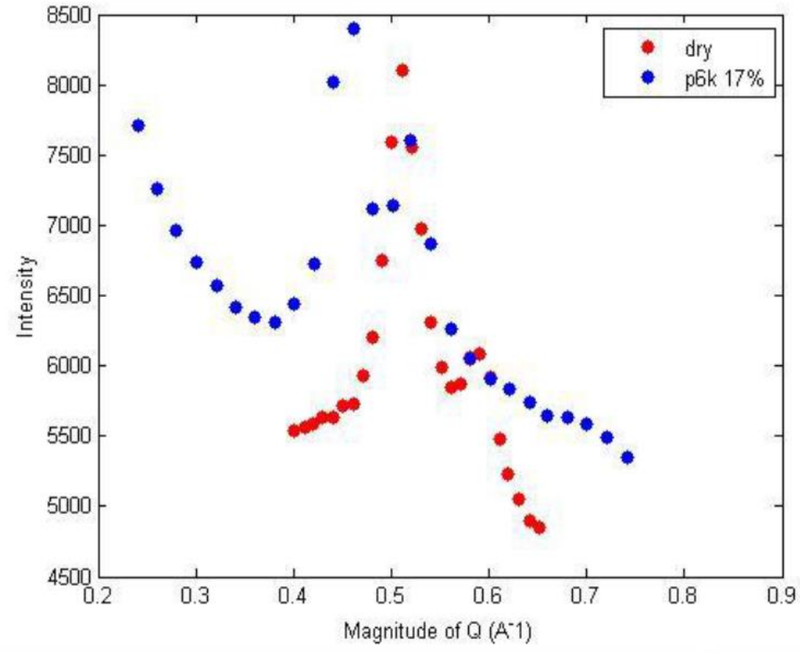
0.022
0.02
0.018
0.016
0.014
0.012
0.01
I_0 (arbitrary units)
0.7
0.6
0.5
0.4
0.3
0.2
Γ_0 ($Å^{-1}$)
300
320
340
360
Temperature (K)

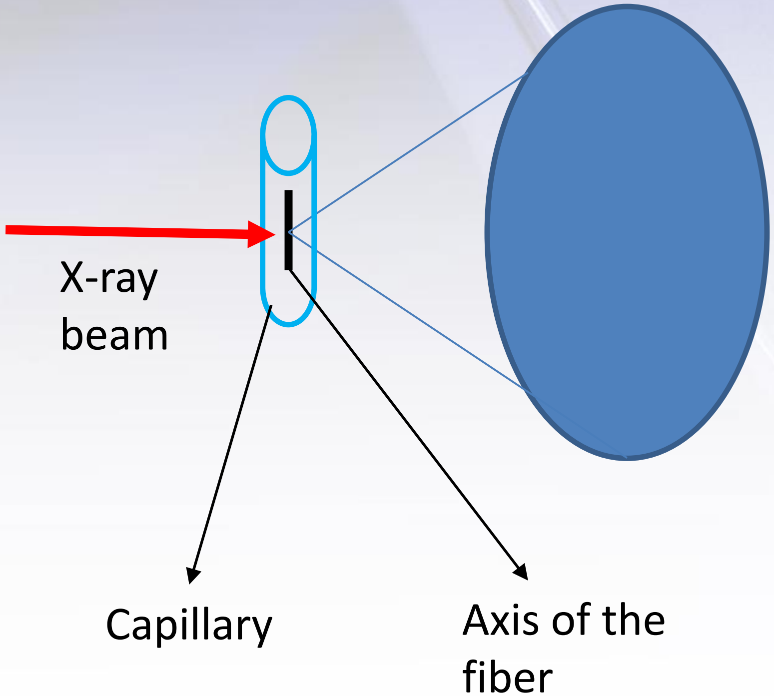

200
100
0
$(\mathcal{L}_p/\zeta(D))$
25
35
45
55
D, Å
9.5
7.5
5.5
log Π, dyne/cm²
II
I
25
35
45
55
D, Å

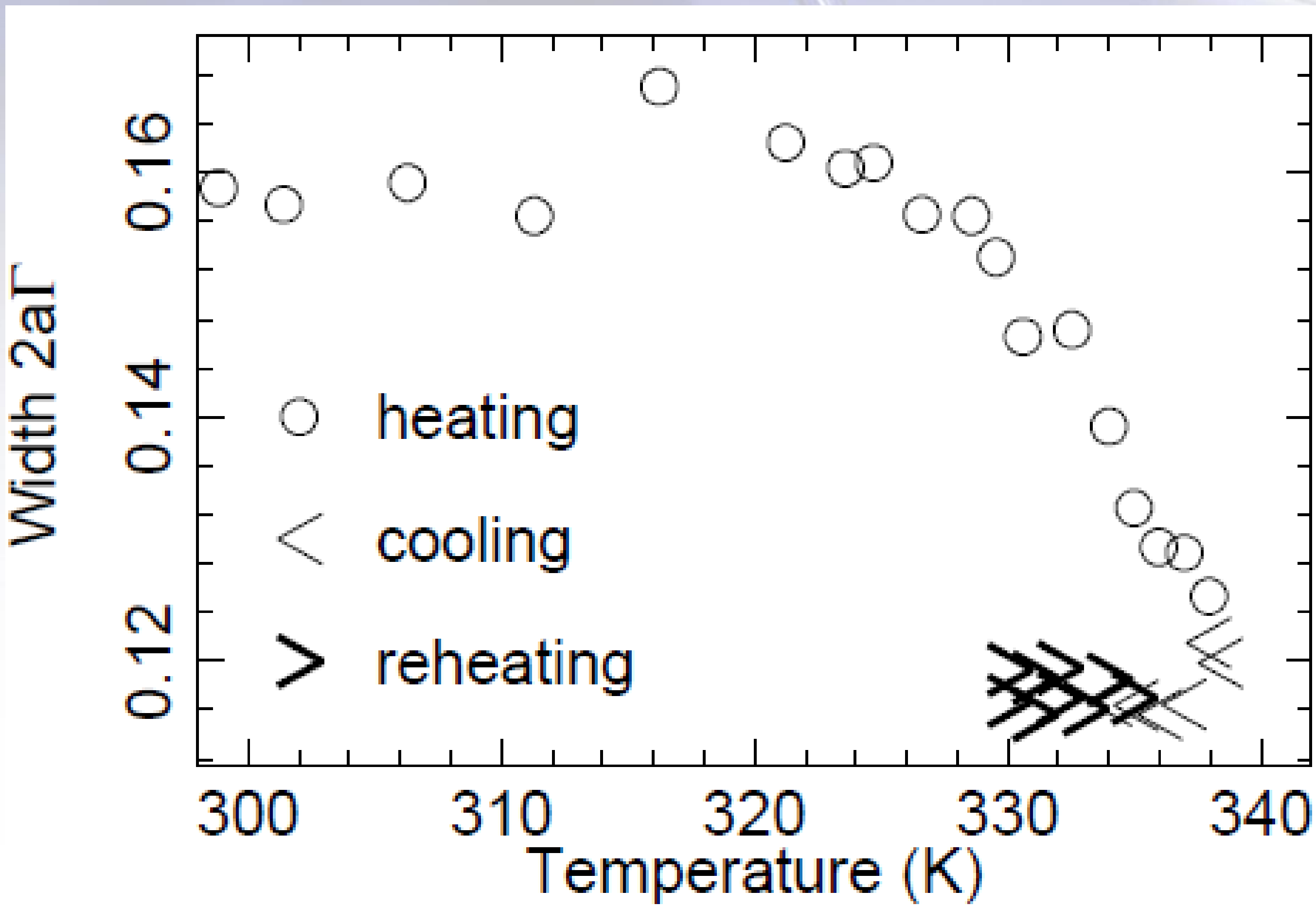



PEG 6k 17% 12−days
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
QL
−0.5
−0.4
−0.3
−0.2
−0.1
0
0.1
0.2
0.3
0.4
0.5
QK


ehtanol 66%
dry
Intensity
Magnitude of Q (A^-1)


dry
p6k 17%
Intensity
Magnitude of Q (A^-1)


X-ray beam
Capillary
Axis of the fiber



Width 2aΓ
0.12
0.14
0.16
heating
cooling
reheating
300
310
320
330
340
Temperature (K)