# SLOW MOLECULAR DYNAMICS CLOSE TO CRYSTAL SURFACES DURING CRYSTALLIZATION OF A PROTEIN LYSOZYME STUIDED BY FCS

# Protein Crystallization

- Protein crystallization is important to determine the 3D structure of protein with the x-ray crystallography

- Complex phase behaviors of protein solutions along with crystallization ( Liquid-liquid phase separation, Random aggregation, Gel or glass formation )

- Metastable and nonequilibrium states

    polycrystalline – spherulites

# Materials - lysozyme

- Existing in Egg-white, Tear, Spit …

- A. Fleming found the function

- Working on immune system

- Attacking chemical bond in a cell wall

- Well-known crystallization process
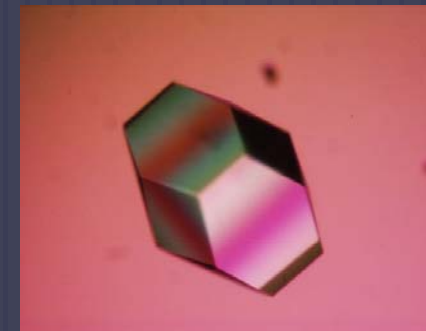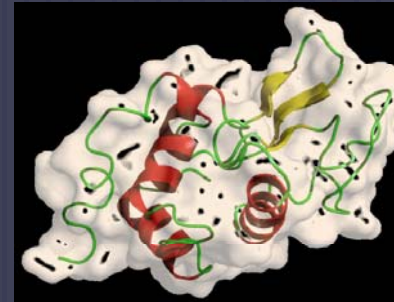
# Materials - lysozyme

- 50 mM Sodium acetate ( $C_2H_3NaO_2$ ) buffer, pH ~ 4.5, High NaCl concentration ( for exhibiting liquid-liquid phase separation )

- 6000 rpm centrifuge 2 min, supernatant

- Sample thickness : 3 μm
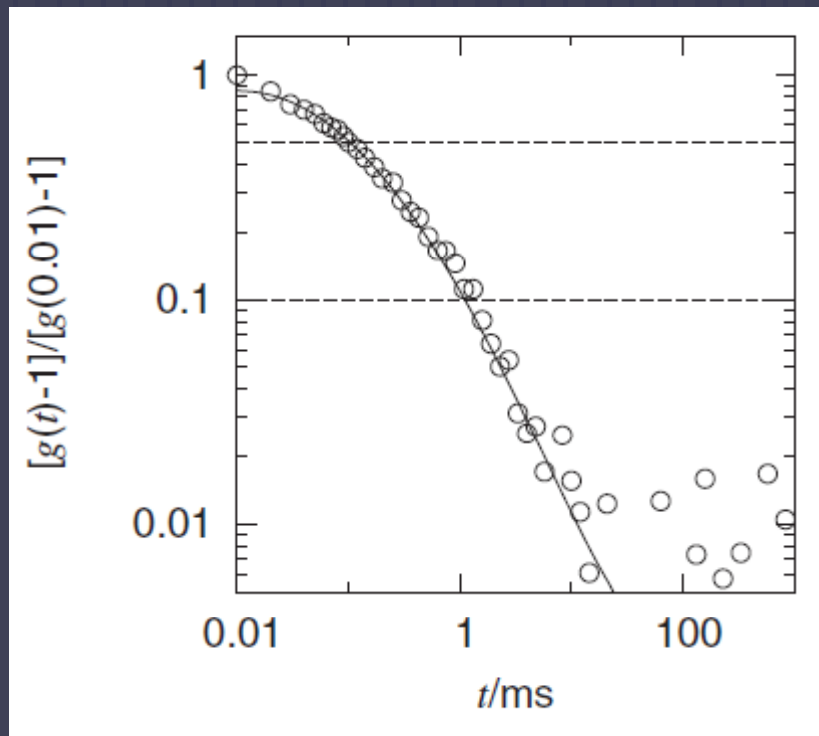
- Alexa-Fluor 488 5-SDP ester labeled lysozyme ~ 1nM

# Model

- 2D-heterogenious diffusion model

$$G(\tau) = \frac{1}{\overline{C} \cdot V}\left(1 + \frac{\tau}{\tau_d}\right)^{-1}\left(1 + \frac{\tau}{\tau_{d,z}}\right)^{-1}$$

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$$G(\tau) = \frac{1}{\overline{C} \cdot V}\left(1 + \left(\frac{\tau}{\tau_d}\right)^{\beta}\right)^{-1}$$

# Diffusion in Bulk Solution



1.0 mg/ml lysozyme solution

$D = 110 \ \mu m^2/s$

$R_{hydro} = 2.0 nm$

# FCS at the surface of a single crystal



Liquid Area

Beam spot

3.7 µm

Crystal Surface

20 µm

Growth rate

5.0 µm / h
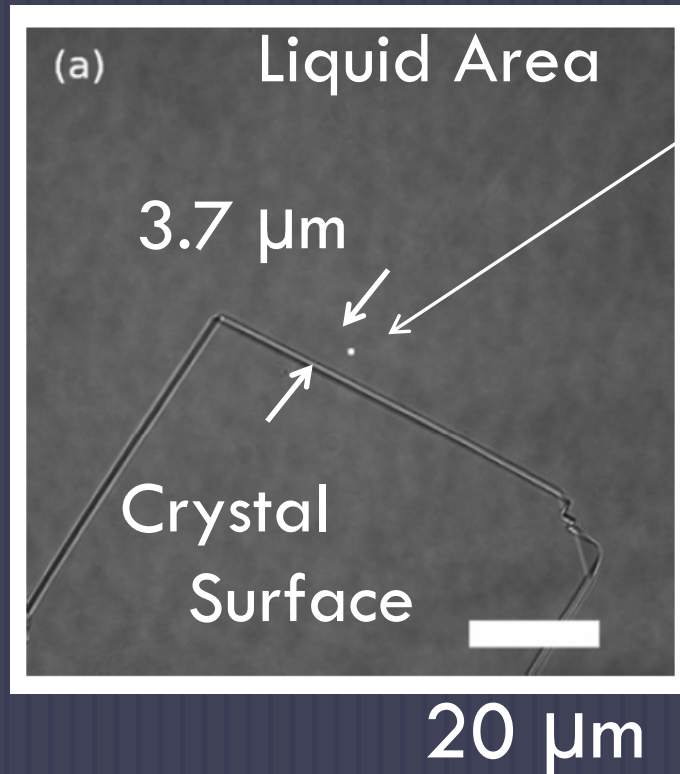
50 mg/ml lysozyme solution

with 0.8M NaCl

# FCS at the surface of a single crystal

## Change of Intensity



Liquid
\+
0
\-
Crystal
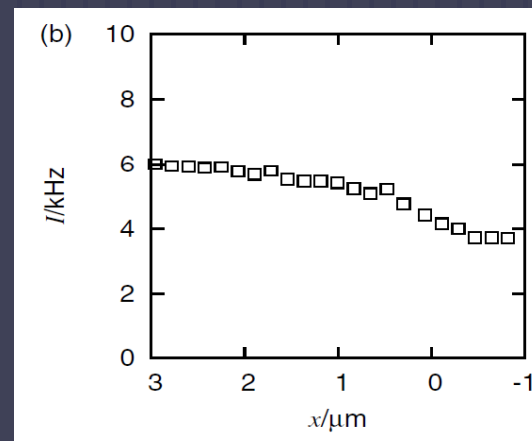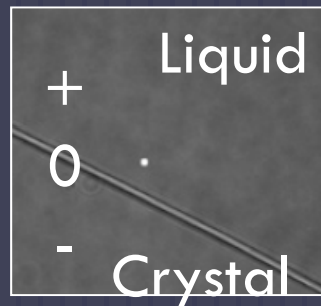




During
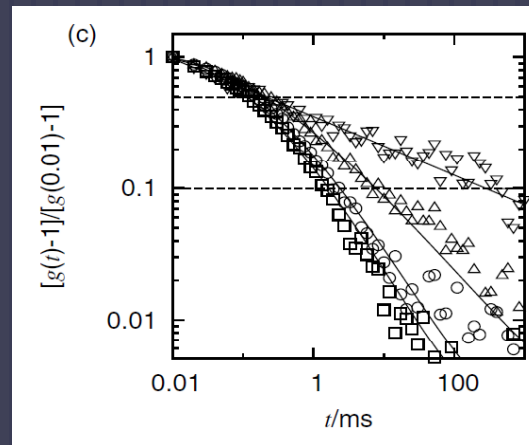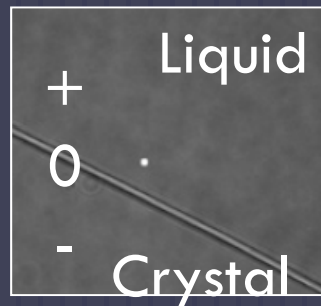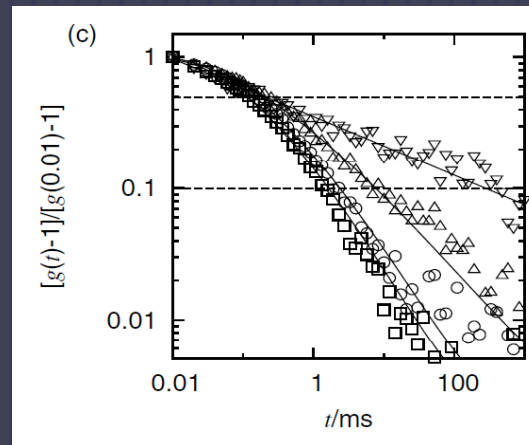Crystal Growth
( metastable )
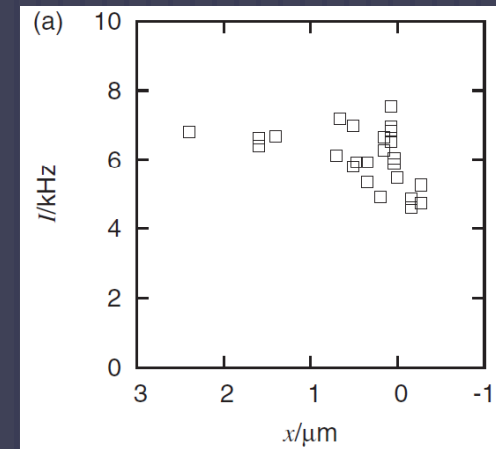
After 2 weeks
( equilibrium ? )

# FCS at the surface of a single crystal
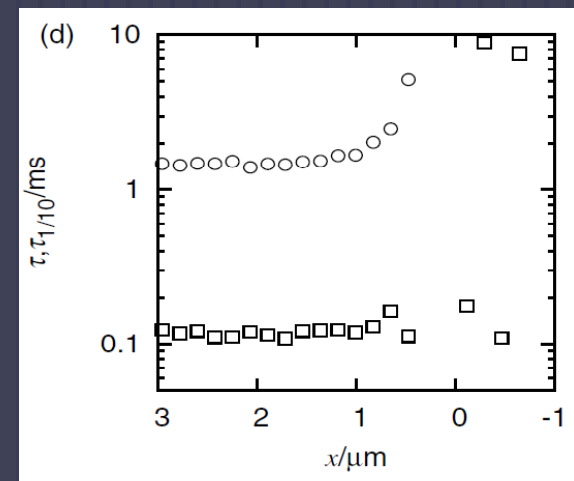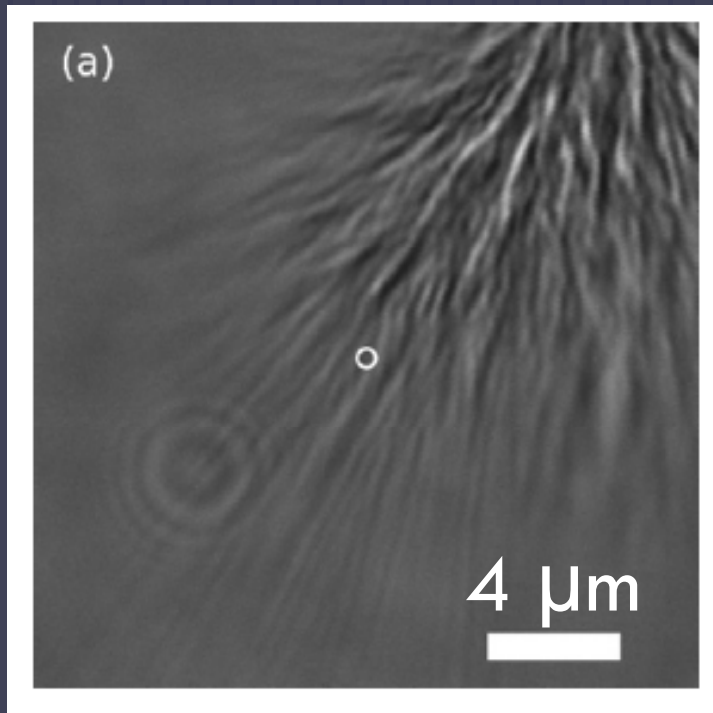
## Molecular Dynamics

# FCS at the surface of a single crystal

## Molecular Dynamics
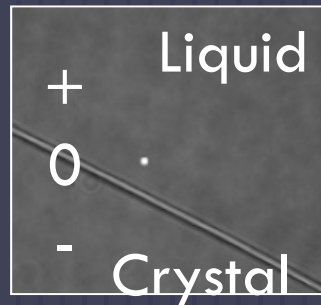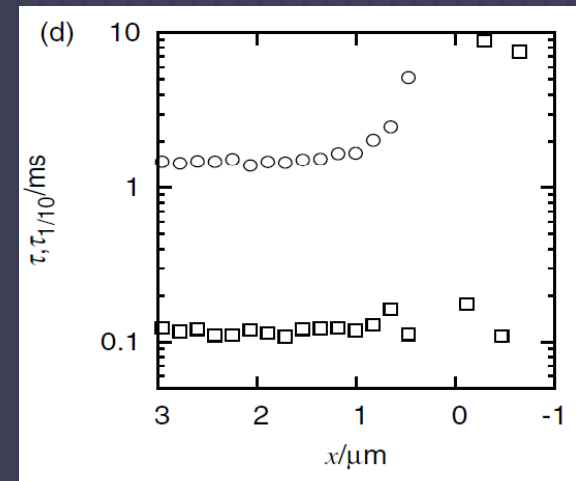
# FCS in a needlelike spherulite



Typical width of the
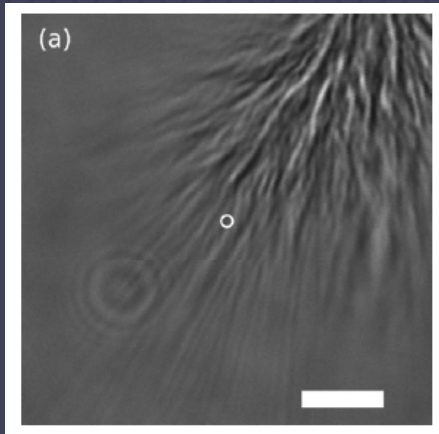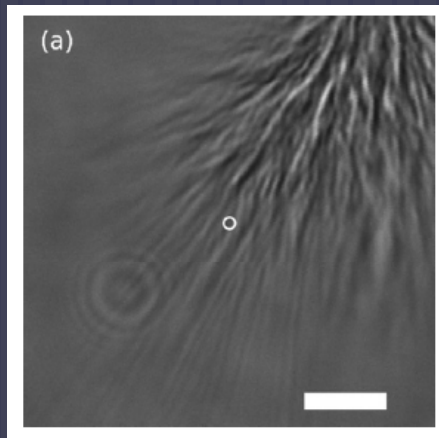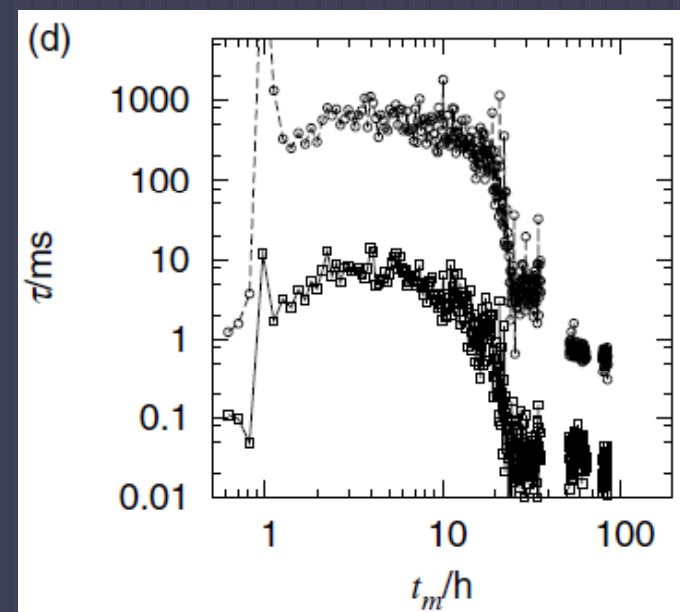    needles ~ 0.1 µm

Growth rate

250 µm / h

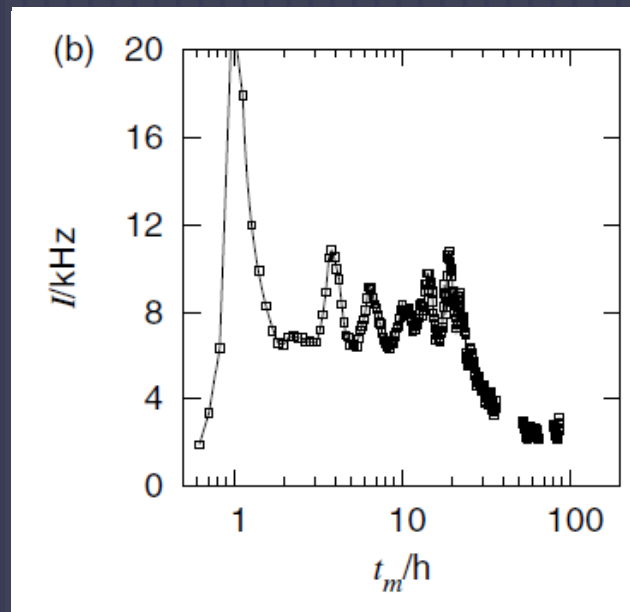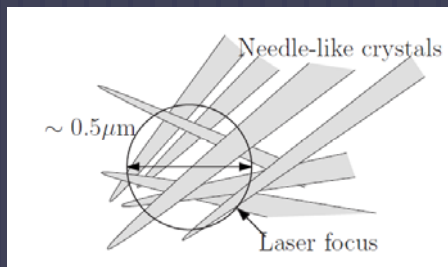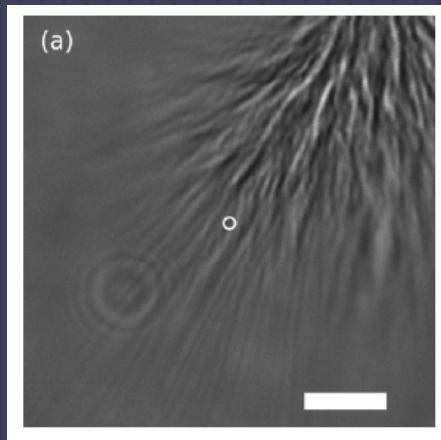50 mg/ml lysozyme solution

with 1.6M NaCl
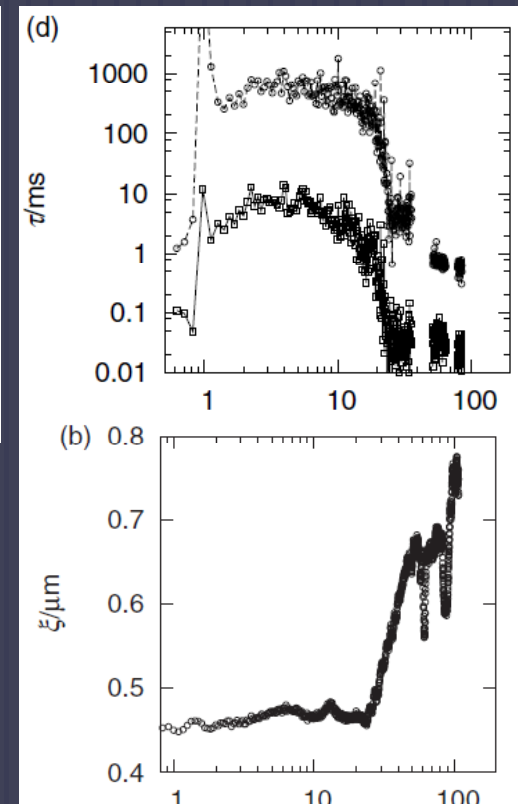
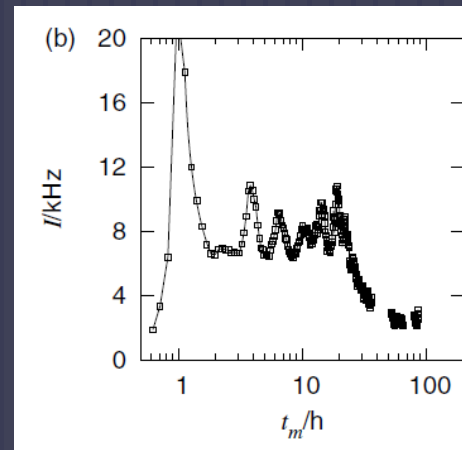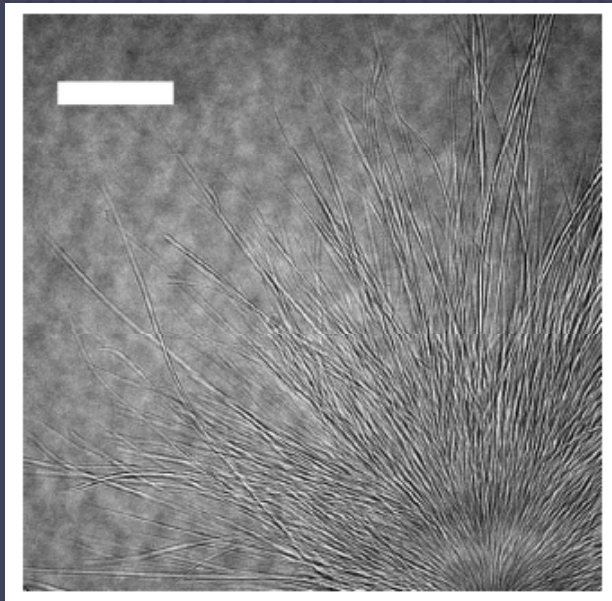# FCS in a needlelike spherulite
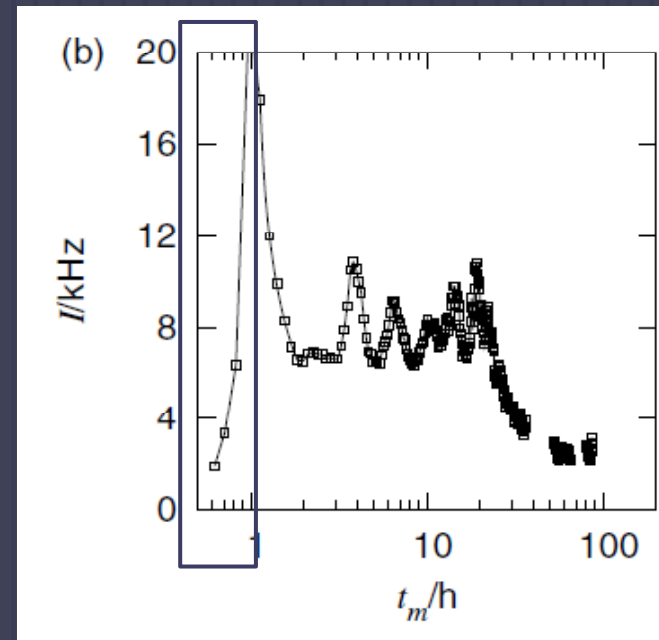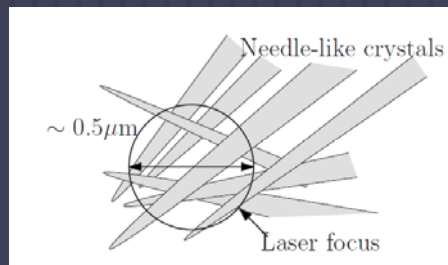
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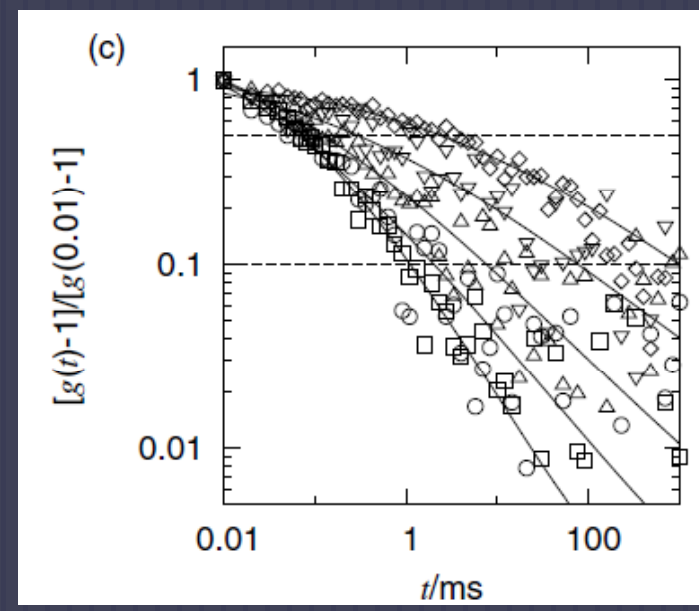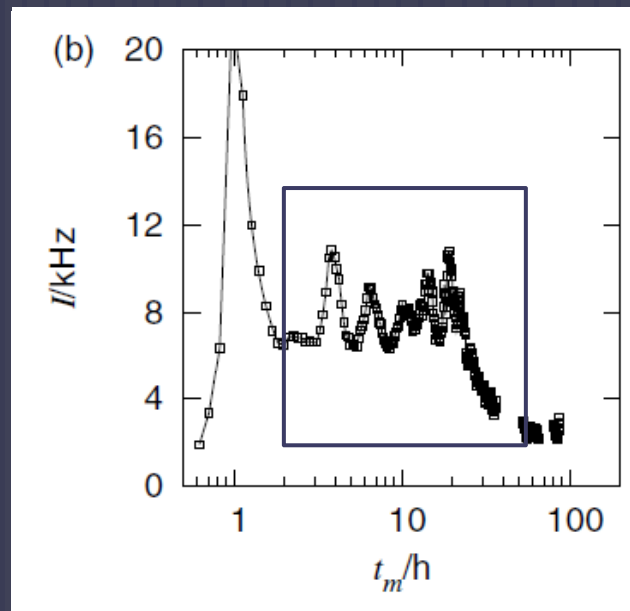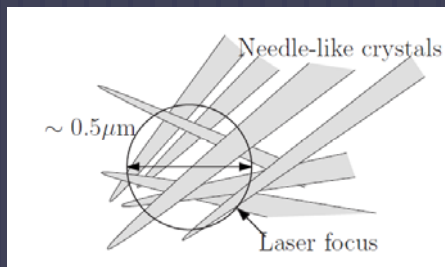
# FCS in a needlelike spherulite

# Change of the internal structure of spherulites

# Conclusion

- Single Crystal

Slow dynamics is originated from the interaction with the surface and molecules. (Clear)

- Needlelike spherulites

Softly connected aggregates are around the needlelike spherulites (non-clear)