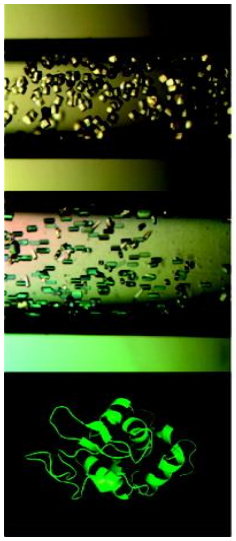
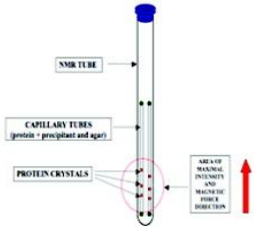
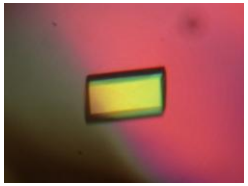
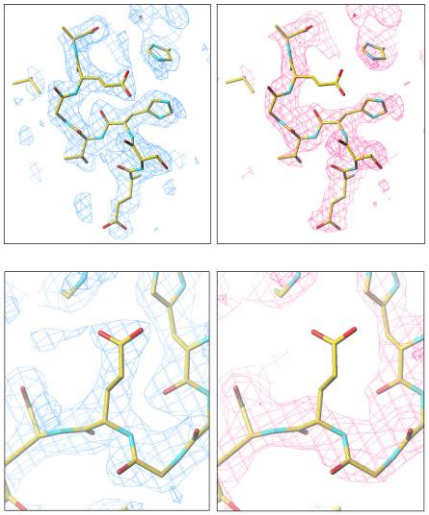
Protein Crystal Quality Enhancement into the Presence of a Strong Magnetic Field







$$\vec{F} = \underbrace{q\vec{E}}_{\text{Electric force}} + \underbrace{qv\vec{} \times \vec{B}}_{\text{Magnetic force}}$$



Dr. Abel MORENO-CARCAMO
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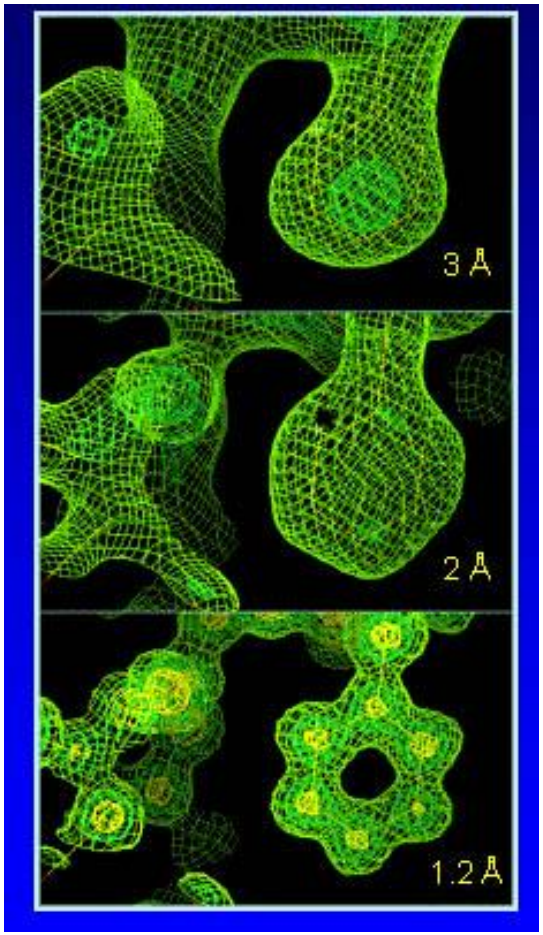
Outline of the talk

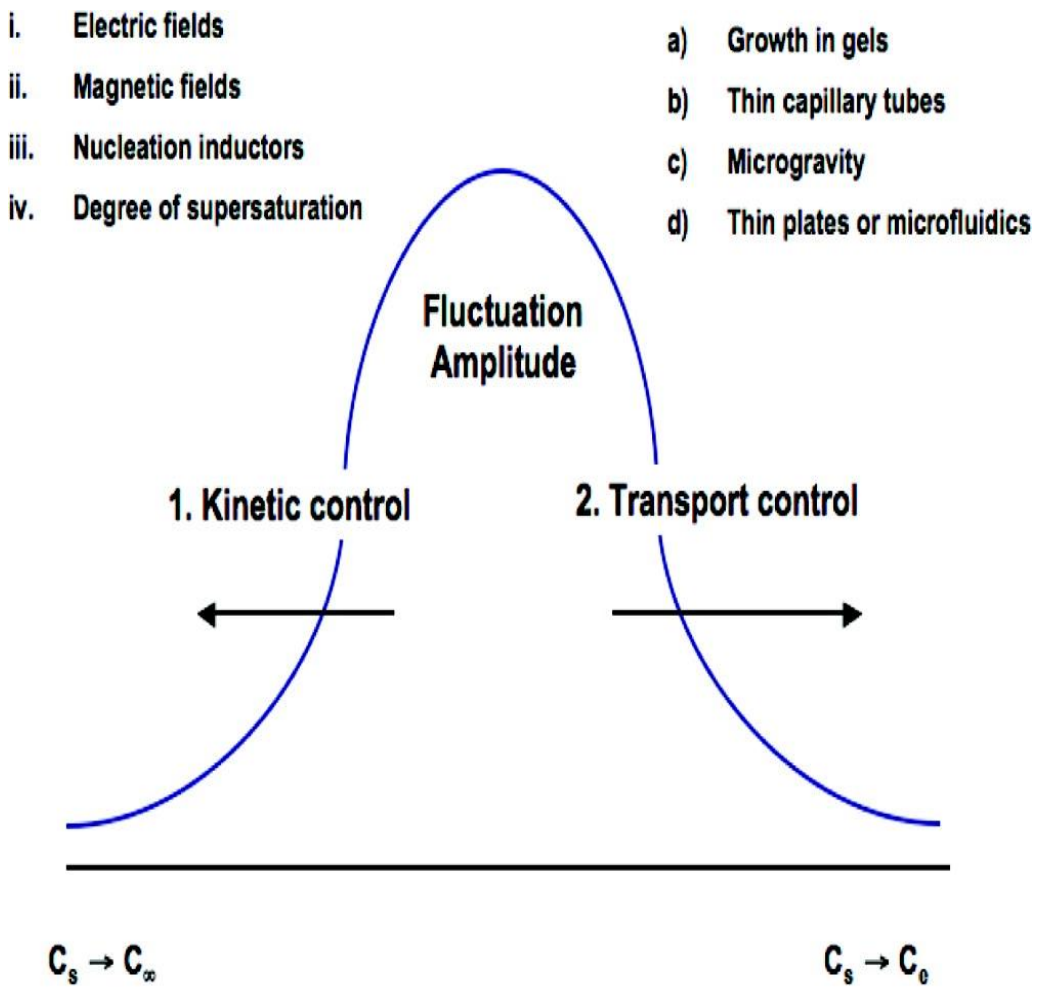
- Historical, Chemical and Physical overview about protein crystallisation (General Introduction).
- The effect of magnetic influence on protein crystallisation process.
- Combining magnetic fields and gel's growth to increase the crystal quality.
- My Master Piece Method on Protein Crystallisation
- Is microgravity necessary?
- Conclusions.

What does crystal quality mean?



High quality single crystals

Ultrapure protein samples



Rate versus transport control or kinetic control of the crystal growth process (adapted from Vekilov et al. reference 32) The right-side of the figure shows different methods for increasing the crystal quality by transport control, while the left-side lists some of the approaches that can influence or affect the kinetics of the process.

Published in: Sachin Surade; Takashi Ochi; Daniel Nietlispach; Dima Chirgadze; Abel Moreno; *Crystal Growth & Design* **2010,** 10, 691-699.

Physical parameters used to induce the nucleation process:

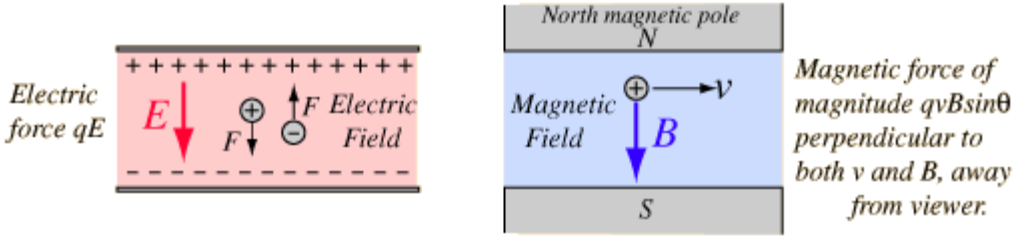
Lorentz Force Law

Both the electric field and magnetic field can be defined from the Lorentz force law:

$$\vec{F} = q\vec{E} + q\vec{v} \times \vec{B}$$

Electric force — Magnetic force

The electric force is straightforward, being in the direction of the electric field if the charge q is positive, but the direction of the magnetic part of the force is given by the right hand rule.



DOES THE MAGNETIC FIELD AFFECT THE CRYSTAL QUALITY AND 3D STRUCTURE OF PROTEINS?

Collaborators:

Prof. Kazuo Nakajima Institute for Materials Research. Tohoku University.
&
Dr. Gen Sazaki. Center for Interdisciplinary Research. Tohoku University, Japan.

Prof. Richard Giegé, Dr. Bernard Lorber, Dr. Claude Sauter. IBMC-CNRS FRANCE.

Dr. Nurit Mirkin, Hunter College, New York (USA)

CONTENTS OF MAGNETIC FIELD EFFECTS

1. Introduction of magnetic field effects

Pioneering works

2. Effects on protein crystallization

1) Overviews
2) Magnetic orientation of crystals
3) Growth rate of crystals
4) Damping of buoyancy convection
5) Quality of crystals
6) Mechanisms of improvement of crystal quality
7) Other effects (number and shape of crystals)

Magnetic orientation of biological macromolecules

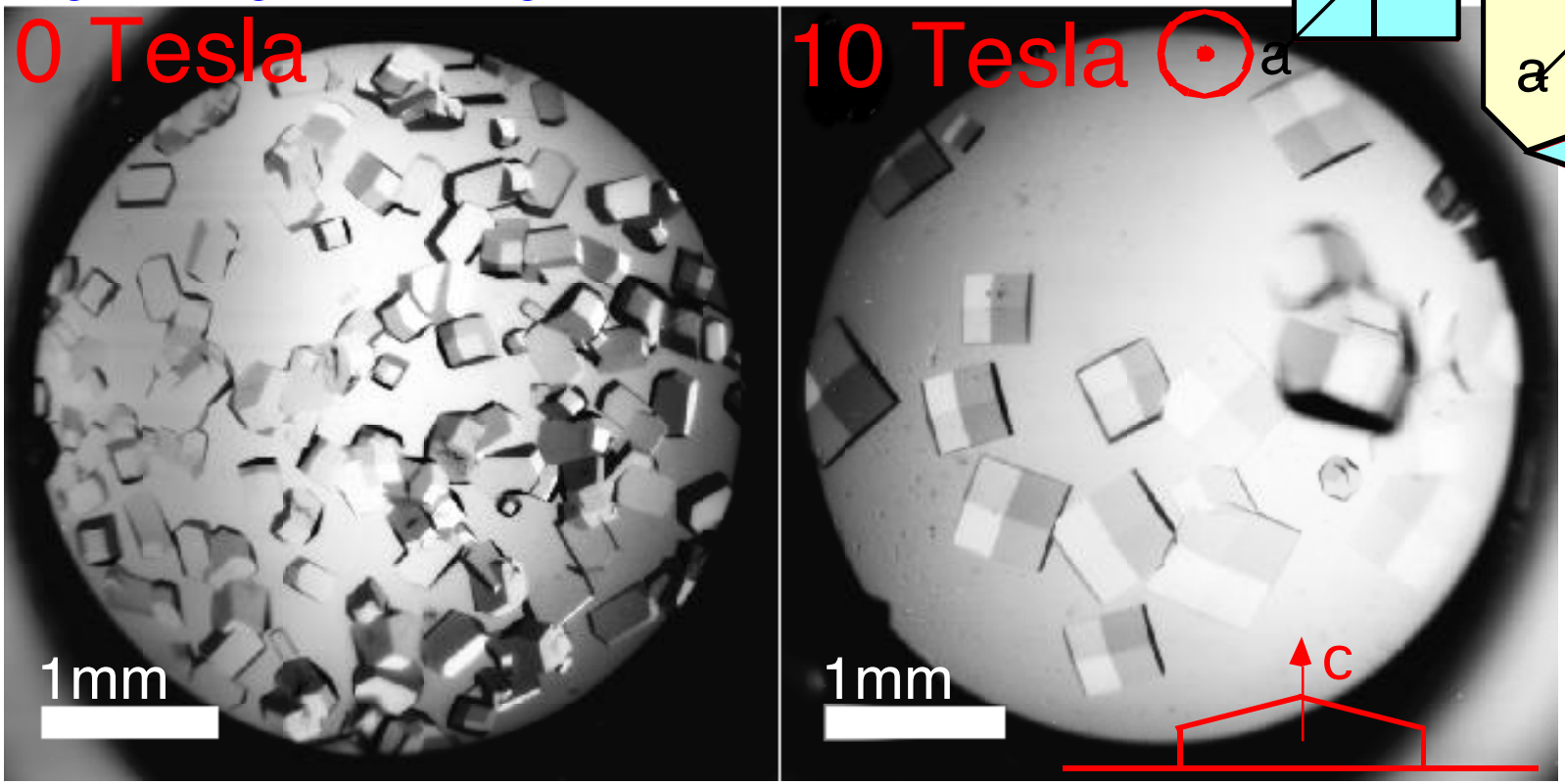
Polyamino acid	Y. Go, et al., Biochim Biophys. Acta, 175, 454 (1969). E.T. Samulski and H.J.C. Berendsen, J. Chem. Phys., 56, 3920 (1972). E.G. Finer and A. Darke, J. Chem. Soc. Faraday Trans. Sect. 1, 71, 984 (1975).
Rhodopsin (Protein)	M. Chabre, Proc. Natl. Acad. Sci. USA, 75, 5471 (1978).
Fibrin (Protein)	A. Yamagishi, et al., J. Phys. Soc. Jpn., 58, 2280 (1989). [3] A. Yamagishi, J. Magnetism Magnetic Materials, 90 & 91, 43 (1990) A. Yamagishi, et al., Physica B, 164, 222 (1990)
DNA	M. Suzuki and H. Nakamura, Proc. Jpn. Acad. B, Phys. Biol. Sci, 71, 36 (1995).
Virus particles	M. Hirai, et al., Phys. Rev. E, 51, 1263 (1995).

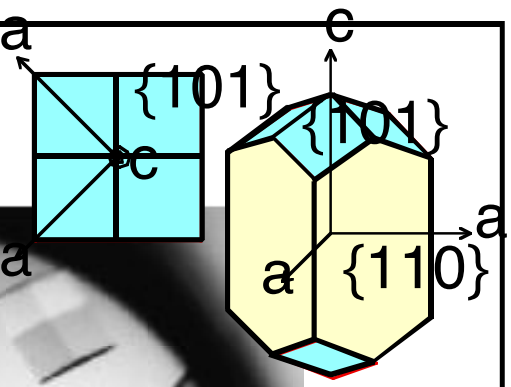
All of them focused only on magnetic field effects on the orientation of one molecule.

Sazaki et al., 1997; Ataka et al., 1997; Wakayama et al., 1997 started the pioneering investigation of the influence of the magnetic field upon the nucleation, crystal growth and crystal quality of biological macromolecules.

Summary of the magnetic field

Lysozyme crystals





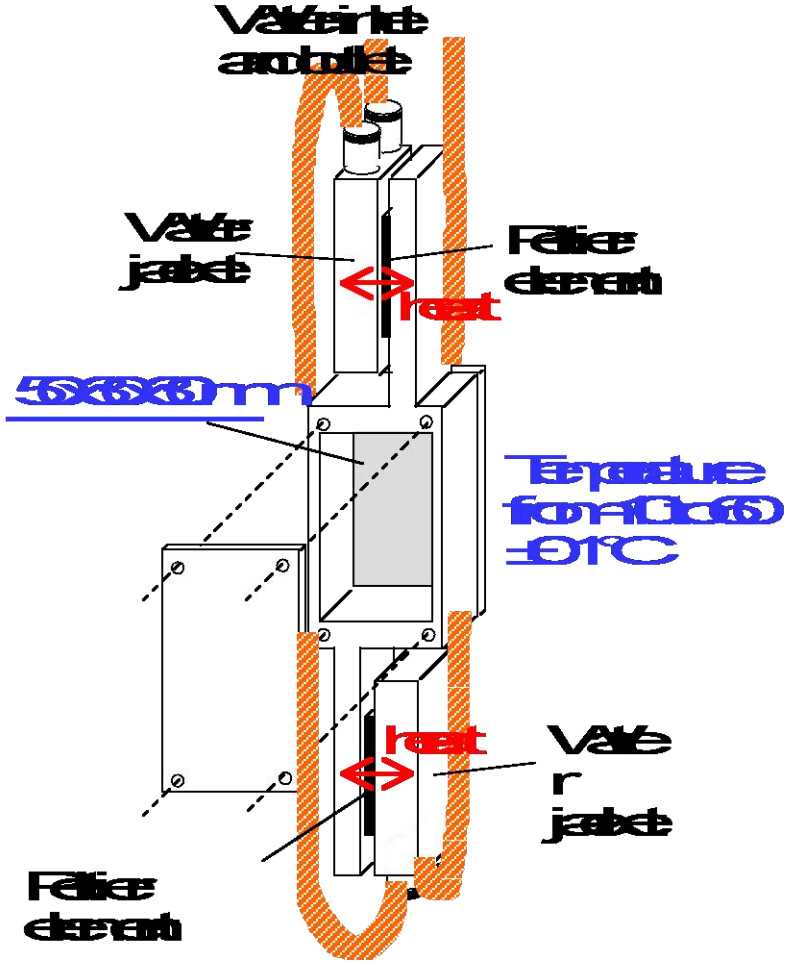
Initial lysozyme conc. 100 mg/ml, Crystallization time 3 days

Magnetic field effects:

1) number of the crystal ↘, 2) magnetic orientation,
3) habit change, 4) growth/dissolution rates ↘,
5) convection ↘, 6) quality of the crystal ↗.

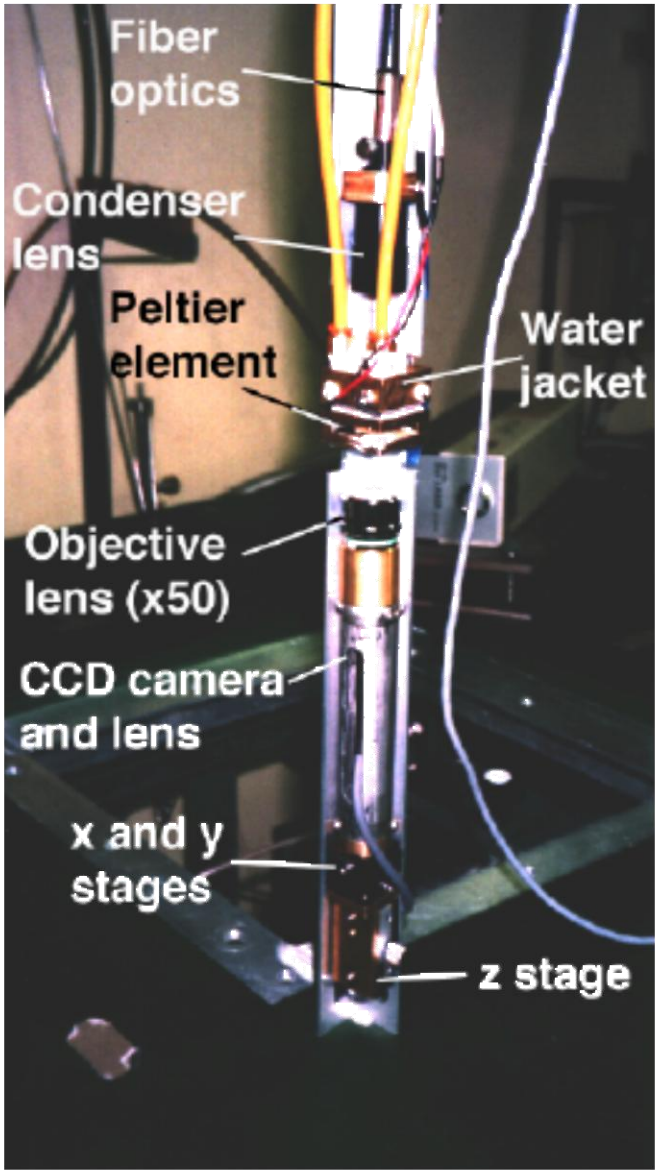
Superconducting magnet system

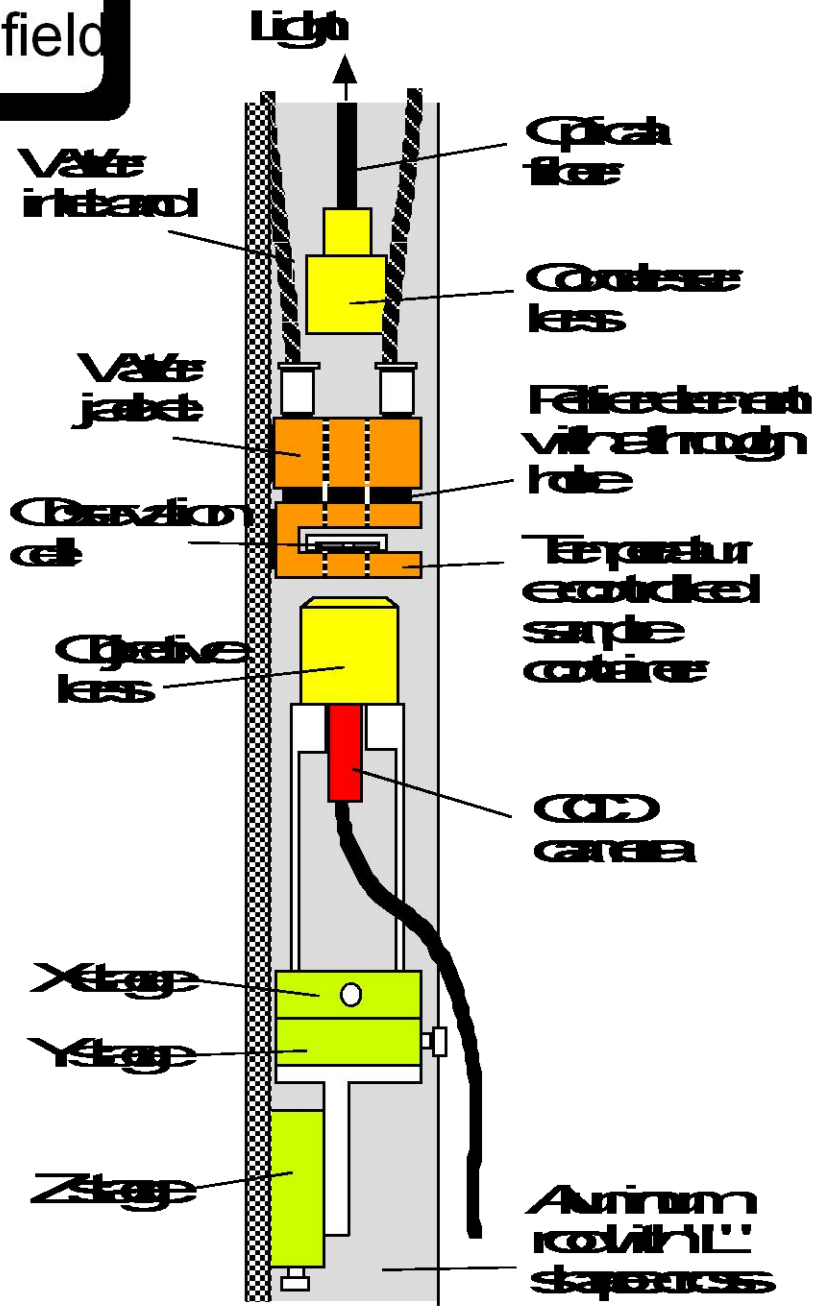
Superconducting magnet of 10T and 100 mm bore type. Direction of the field can be set freely.



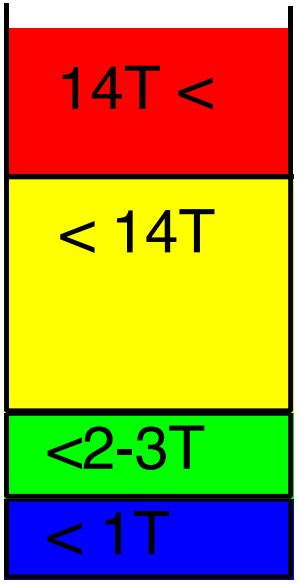
Temperature controlled chamber using Peltier elements

Optical microscope for in-situ observation under high magnetic field





Strength of a magnetic field



Hybrid magnet

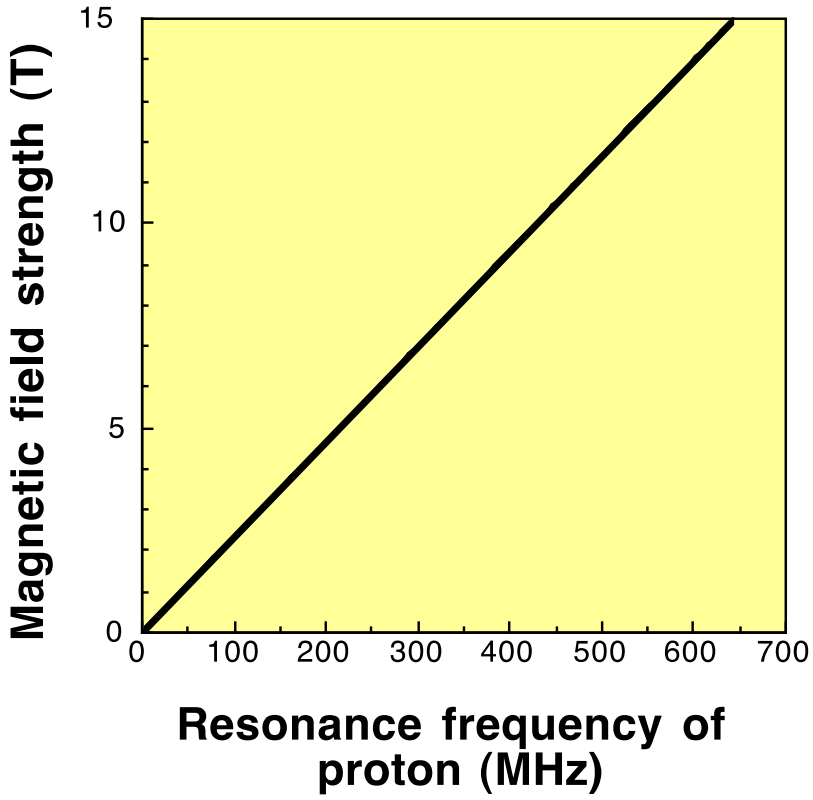
Superconducting magnet

Electric magnet

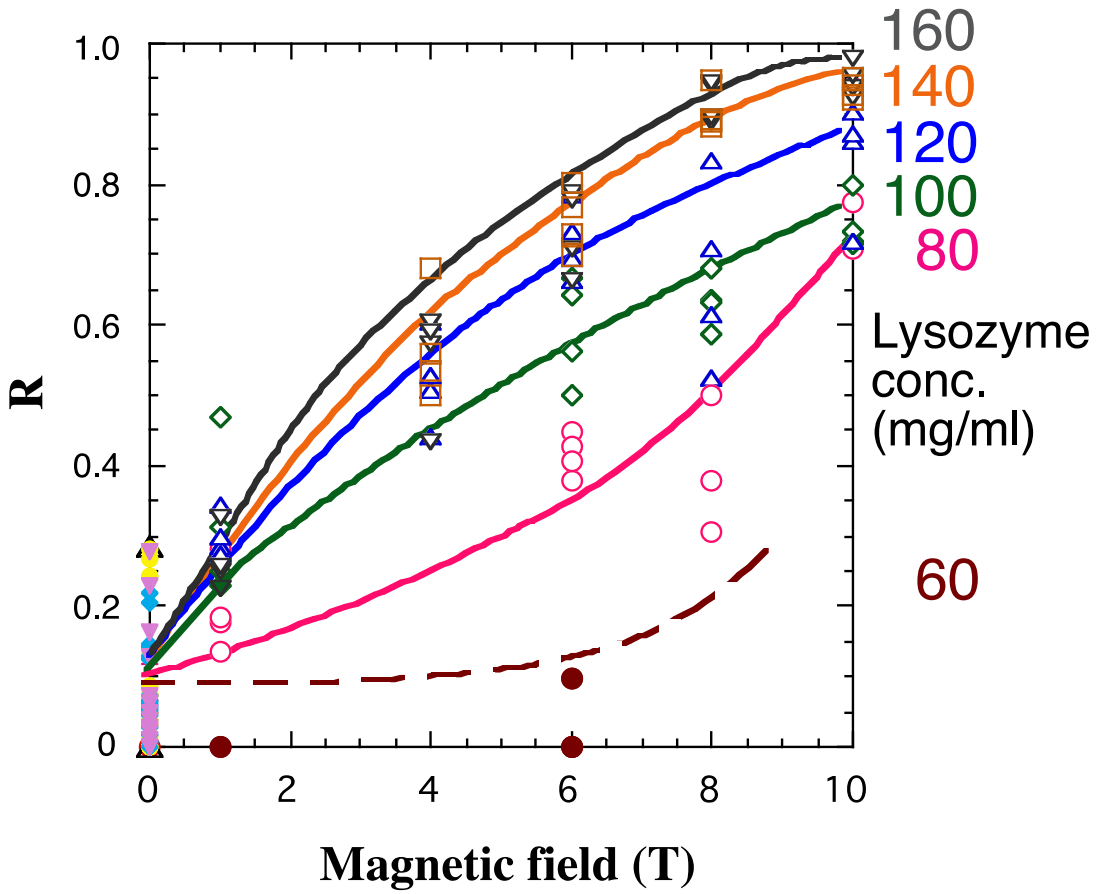
Permanent magnet
(ordinal magnet ~ 0.2-0.3T

1 T = 10,000 gauss

Comparison with a proton NMR



Orientation of the crystal



Deffinition of orientation ratio R

$$R = \frac{\text{Number of oriented crystals}}{\text{Total number of crystals}}$$

Magnetic field ↗

↓

Orientation ↗

Effects was observed even at 1T.

Lysozyme concentration ↗

↓

Orientation ↗

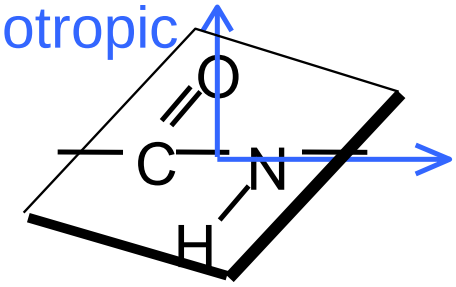
Magnetic anisotropy of protein molecule

Protein molecule is **diamagnetic.**

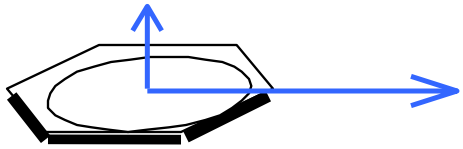
→ (i) Peptide bond in a backbone.
(ii) Aromatic ring in a side chain of amino acid.

1) Peptide bond

Anisotropic

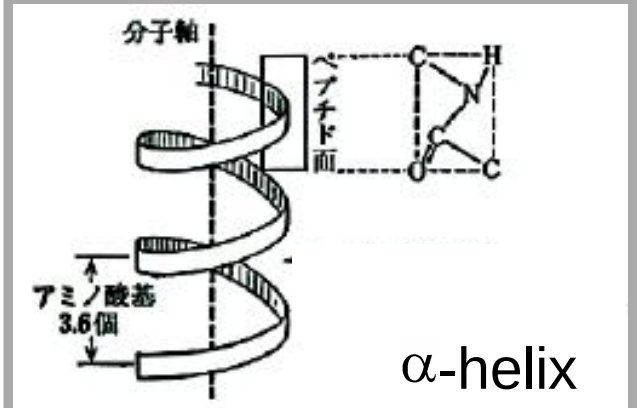


2) Aromatic ring

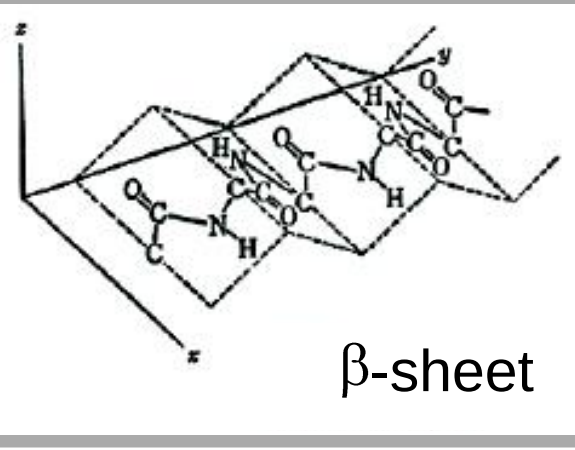


Anisotropy of diamagnetic susceptibility: large

F. Takahashi, "Magnetism and Life, 2'nd ed.", (Tokyo, Gakkai Syuppan Center, 1989) in Japanese.

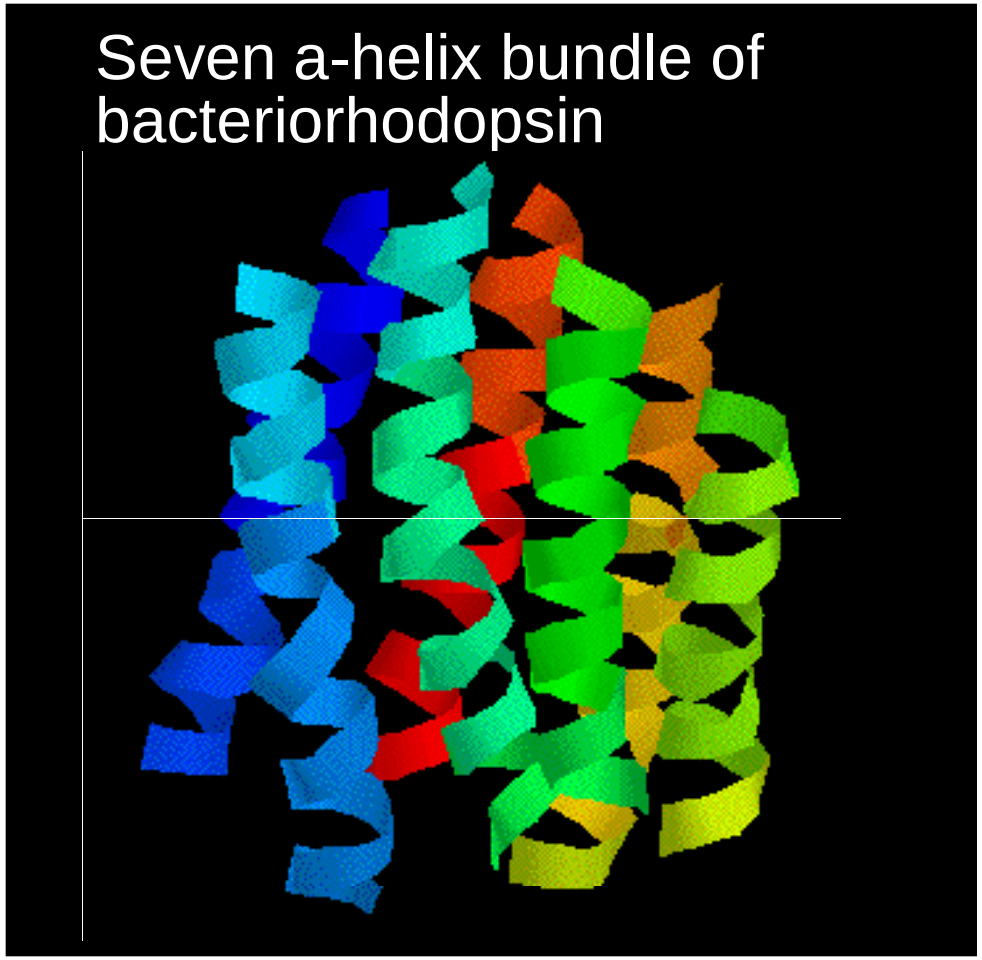


α-helix



β-sheet

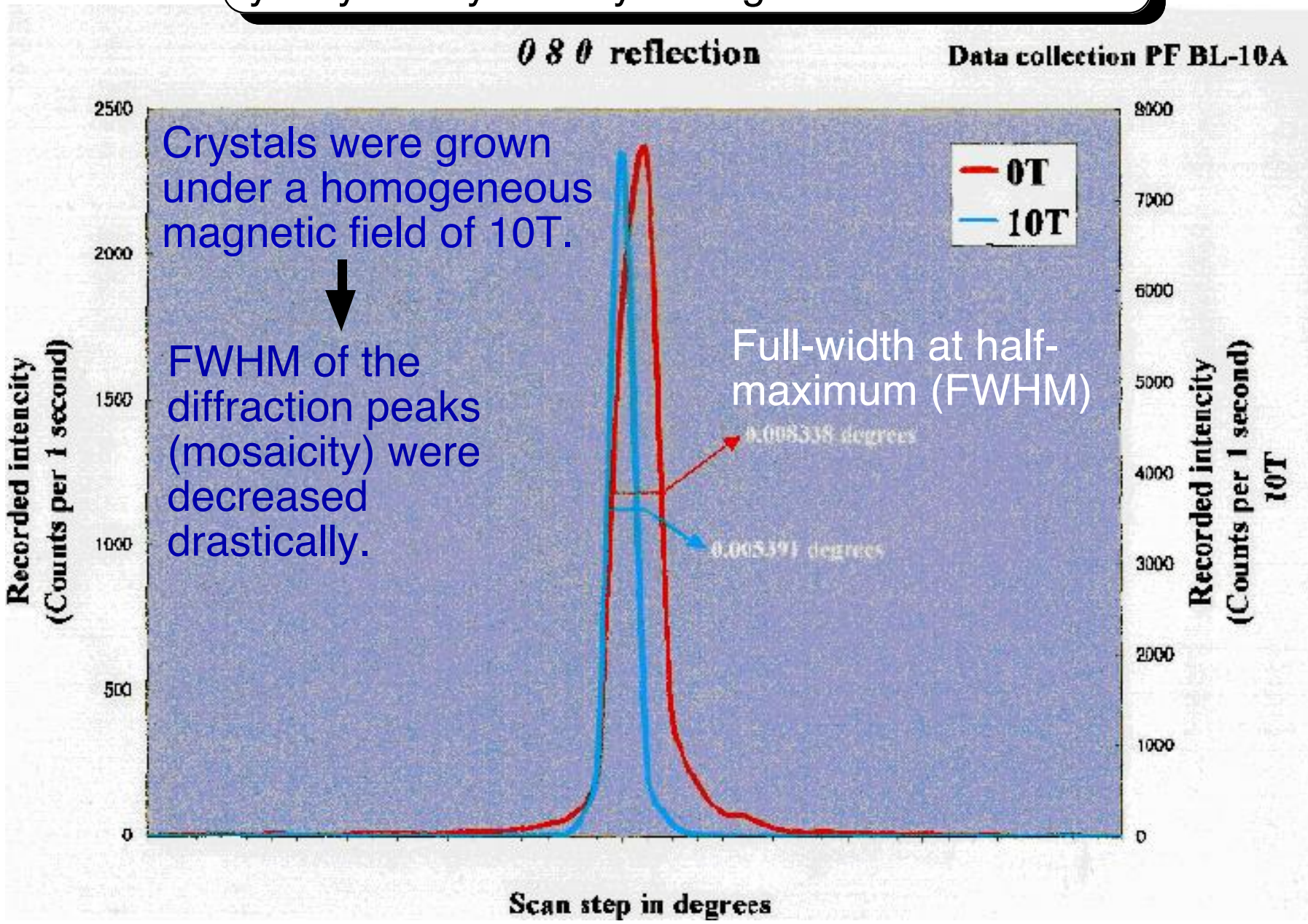
Membrane protein



Since membrane proteins have a very large diamagnetic anisotropy, those proteins are most promising to crystallise under a magnetic field.

Protein Data Bank, 1BAC: K.-C. Chou, L.Carlacci, G.M.Maggiora, L.A. Parodi and M.W. Schulz, *Protein Sci.*, **1** (1992) 810.

Enhancement in the perfection of orthorhombic lysozyme crystals by a magnetic field



Diffraction spots from orthorhombic lysozyme crystals

0 T

10 T

Studies previously published

Homogeneous magnetic field

Orthorhombic lysozyme crystal

T. Sato, Y. Yamada, S. Saijo, T. Hori, R. Hirose, N. Tanaka, G. Sazaki, K. Nakajima, N. Igarashi, M. Tanaka, Y. Matsuura, "Enhancement in the perfection of orthorhombic lysozyme crystals grown in a high magnetic field (10T)", *Acta. Cryst.*, **D50** (2000) 1079-1083.

Homogeneous and inhomogeneous magnetic field

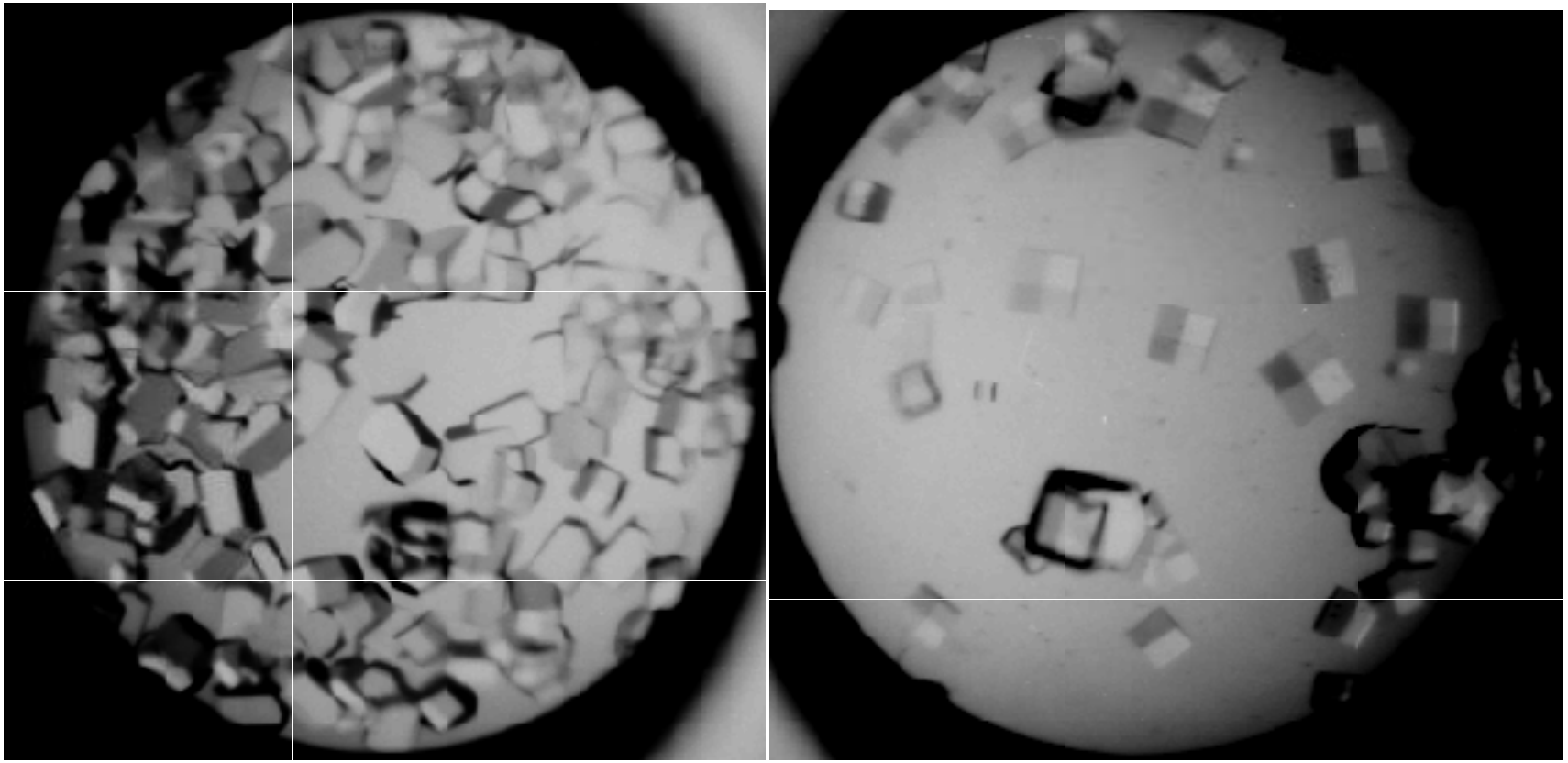
Snake muscle fructose-1, 6-bisphosphatase

S.-X. Lin, M. Zhou, A. Azzi, G.-J. Xu, N.I. Wakayama, M. Ataka, "Magnet used for protein crystallization: novel attempts to improve the crystal quality", *Biochem. Biophys. Res. Commun.*, **275** (2000) 274-278.

Number of lysozyme crystals

0 Tesla

10 Tesla ⊙



Initial lysozyme concentration 100 mg/ml, Crystallization time 3 days.

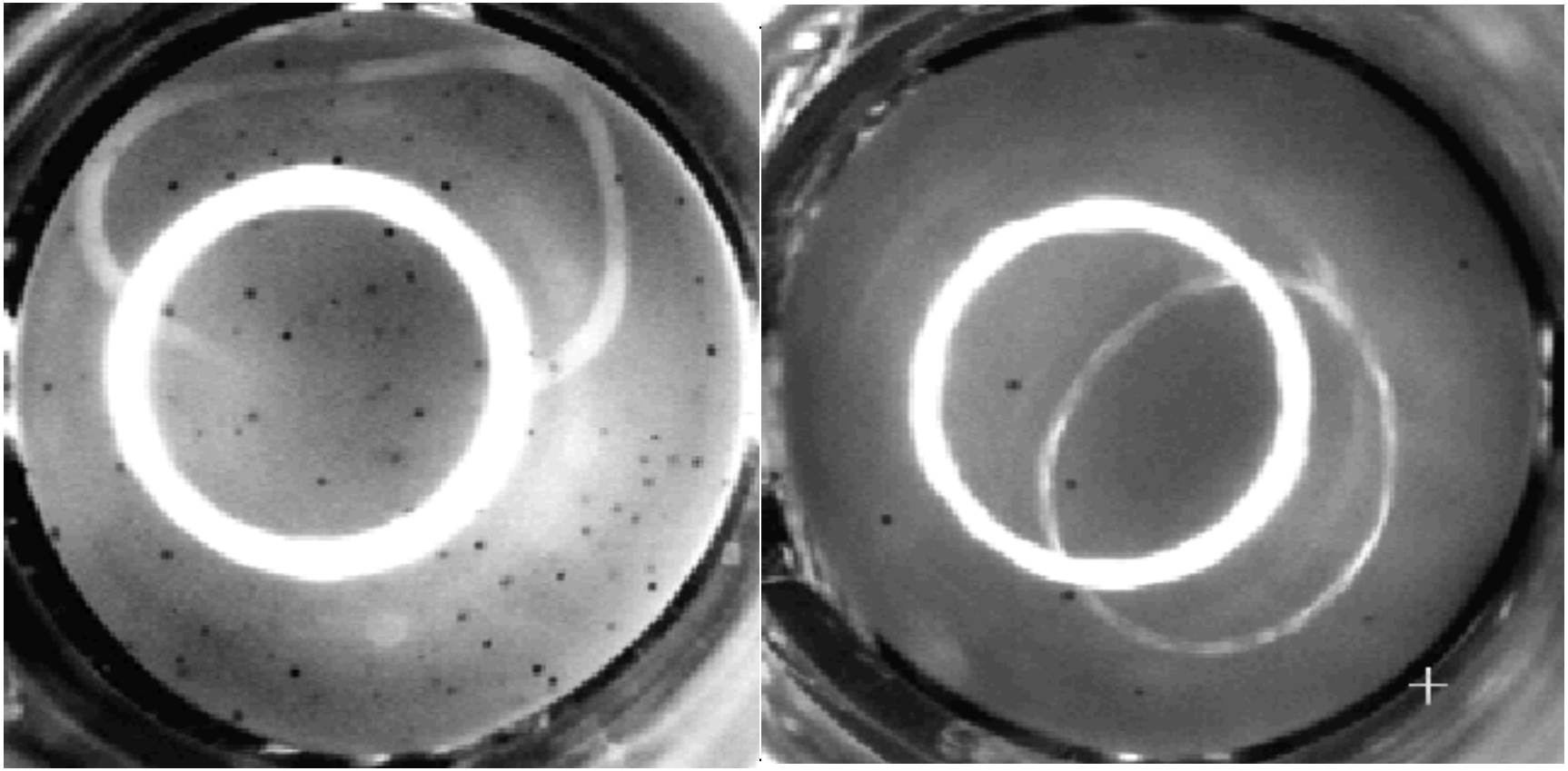
Ref. Sazaki et al. JCG 169 (1996)355-360

1 mm

Number of ferritin crystals

0 Tesla

10 Tesla ⊙



Ferritin 10 mg/ml, $CdSO_4$ 25 mg/ml,
Crystallization time 3 days.

1 mm

Ref. Sazaki et al. JCG 169 (1996)355-360

Summary

What was understood?

1) Magnetic orientation of crystals
   Quantitatively understood.
   Orientation of protein crystals can be controlled.

2) Magnetic damping of buoyancy convection
   Quantitatively understood.

3) Improvement in quality of crystals
   Its mechanisms has been almost understood.
   Practically applied to growth of high quality crystals.

What was not?

4) Magnetic field effects on the nucleation & growth kinetics of protein crystals
   Not understood yet.
   Shape of crystals is also the consequence of the growth kintecs.

Physical parameters used to induce the nucleation process:

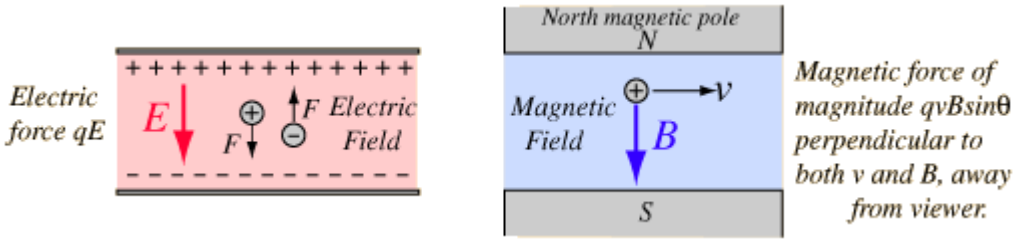
Lorentz Force Law

Both the electric field and magnetic field can be defined from the Lorentz force law:

$$\vec{F} = q\vec{E} + q\vec{v} \times \vec{B}$$

Electric force — Magnetic force

The electric force is straightforward, being in the direction of the electric field if the charge q is positive, but the direction of the magnetic part of the force is given by the right hand rule.

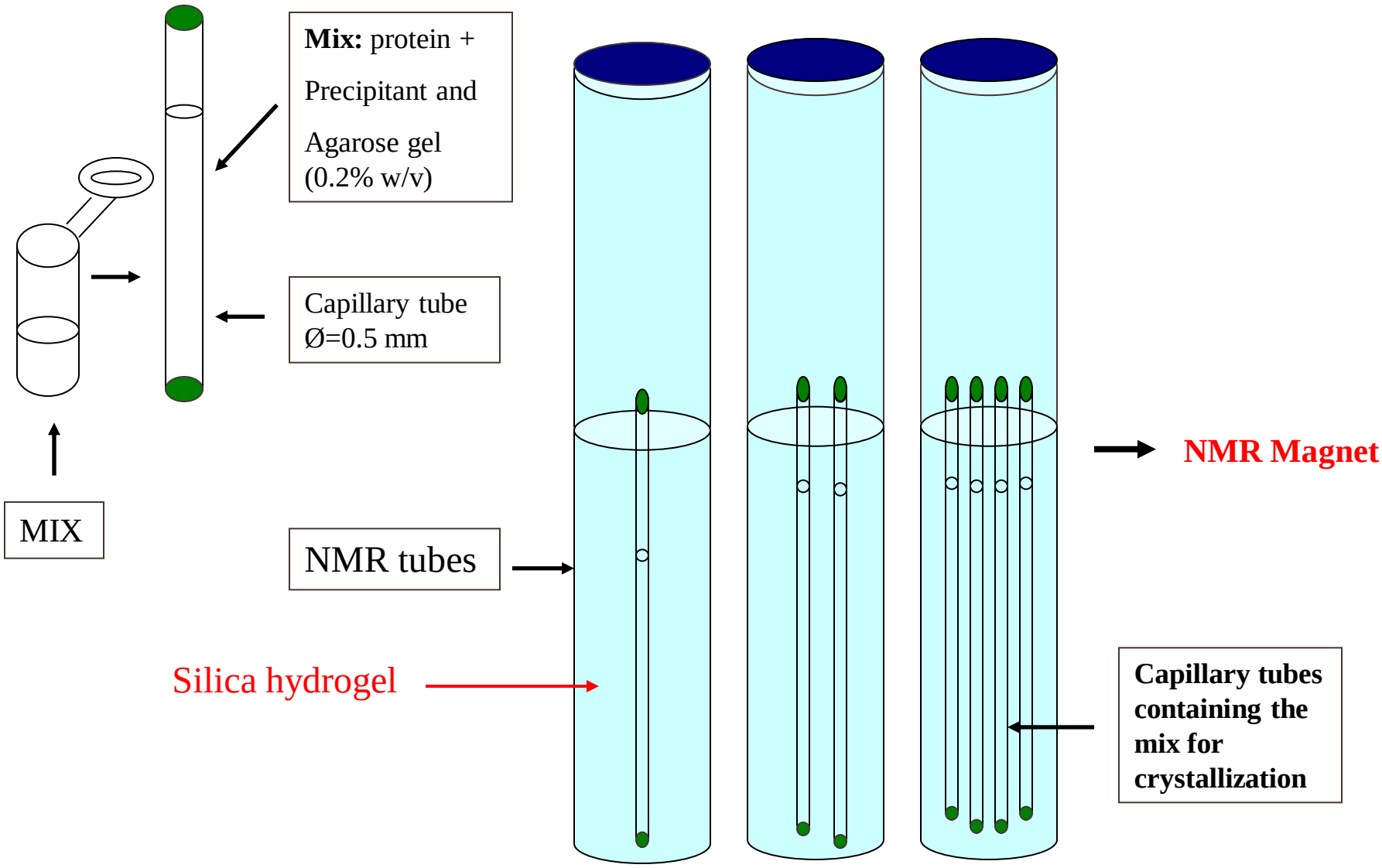


But…Is there a Universal Method to be applied anywhere around the World?

YES, THERE IS!

This method allows to obtain: high quality crystals by coupling magnetic field, kinetics control & gel growth

EXPERIMENTAL SET -UP



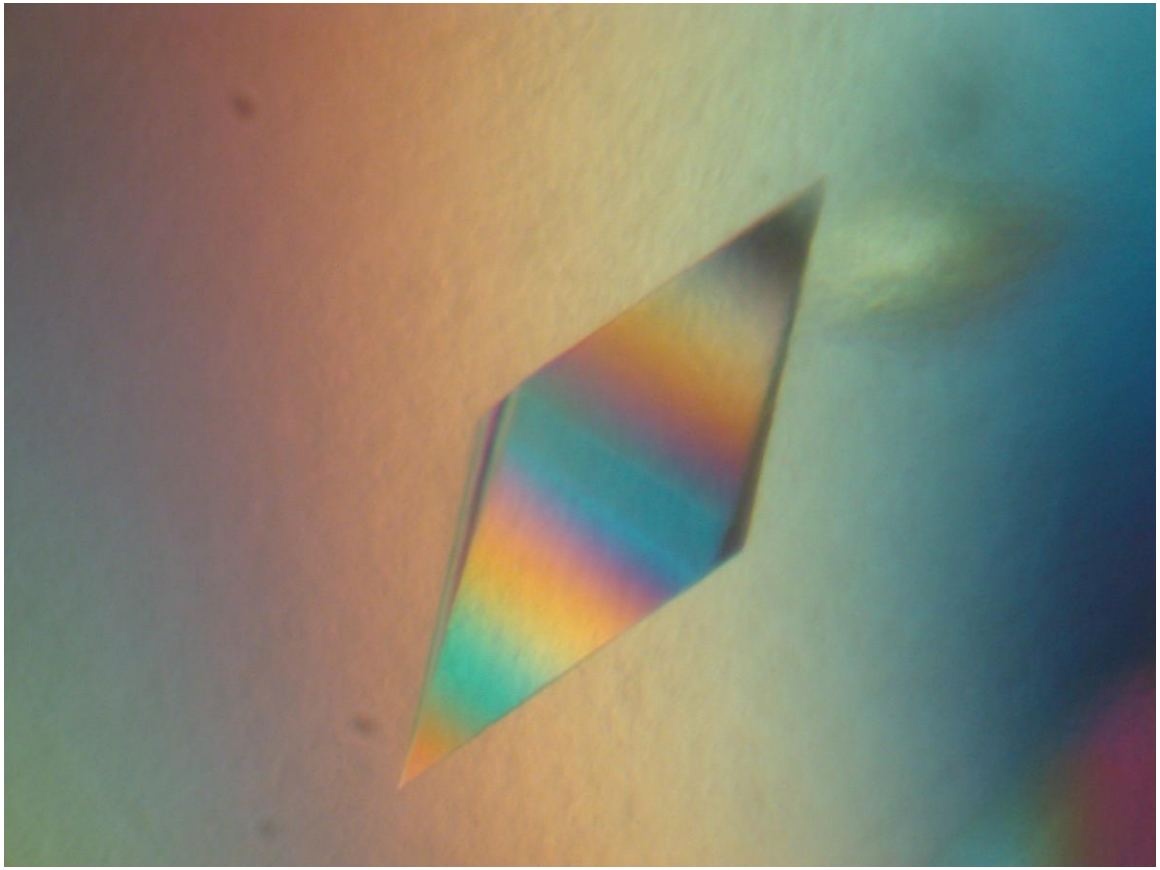
Reference: A. Moreno et al., "***Protein crystal growth in gels and stationary magnetic fields***". Crystal Research and Technology **42** (2007) 231-236

NMR and Crystal Growth in Gels. EXPERIMENTAL SET-UP and FACILITIES

Reference: A. Moreno et al., "***Protein crystal growth in gels and stationary magnetic fields***". Crystal Research and Technology **42** (2007) 231-236

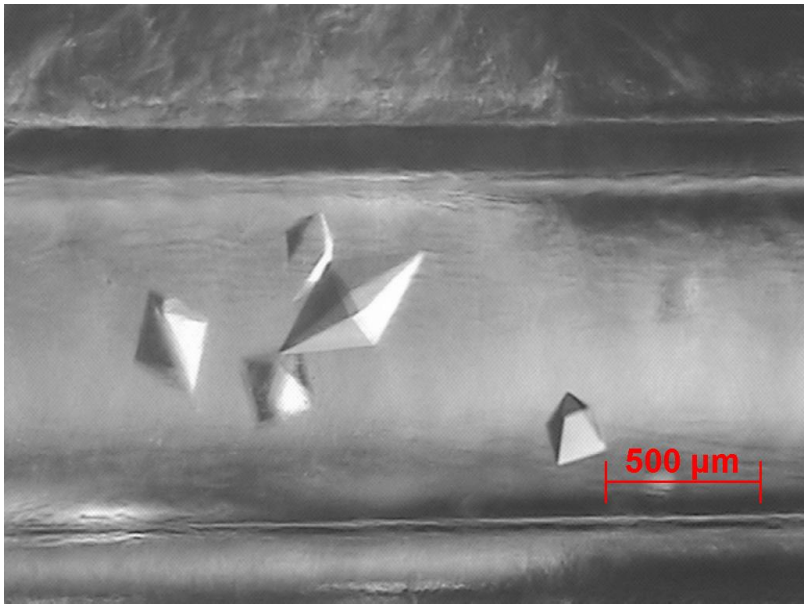
Application to thaumatin from Taumatoccocus daniellii

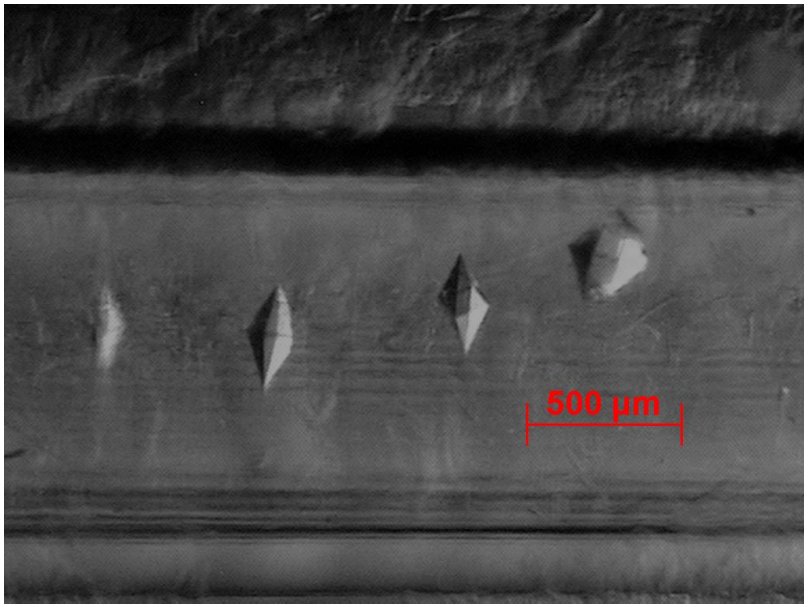
THAUMATIN



MW 22 kDa, pI 12.0 / P.A. NaK Tartrate

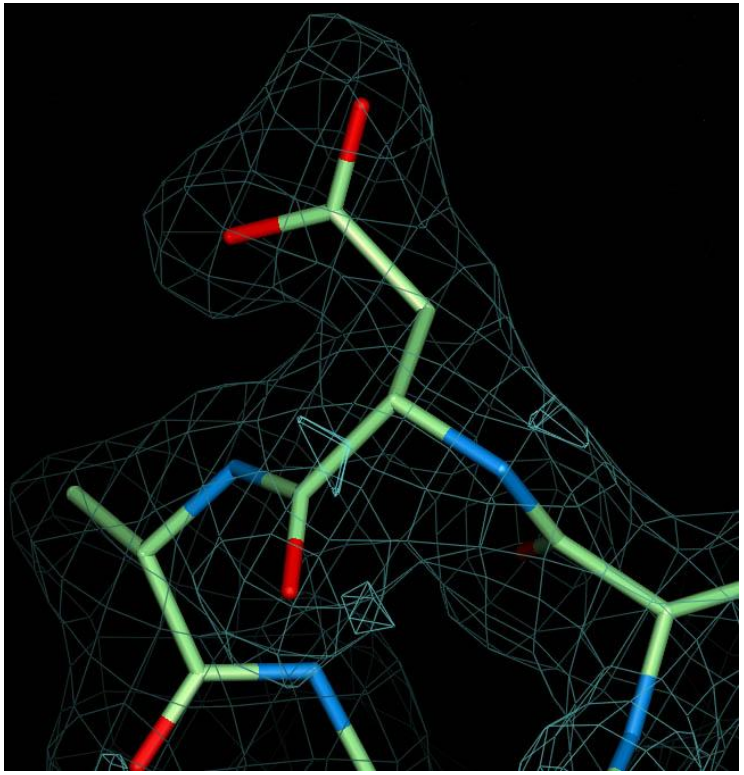
Effect of the magnetic field on the orientation of thaumatin crystals grown in gels inside capillary tubes



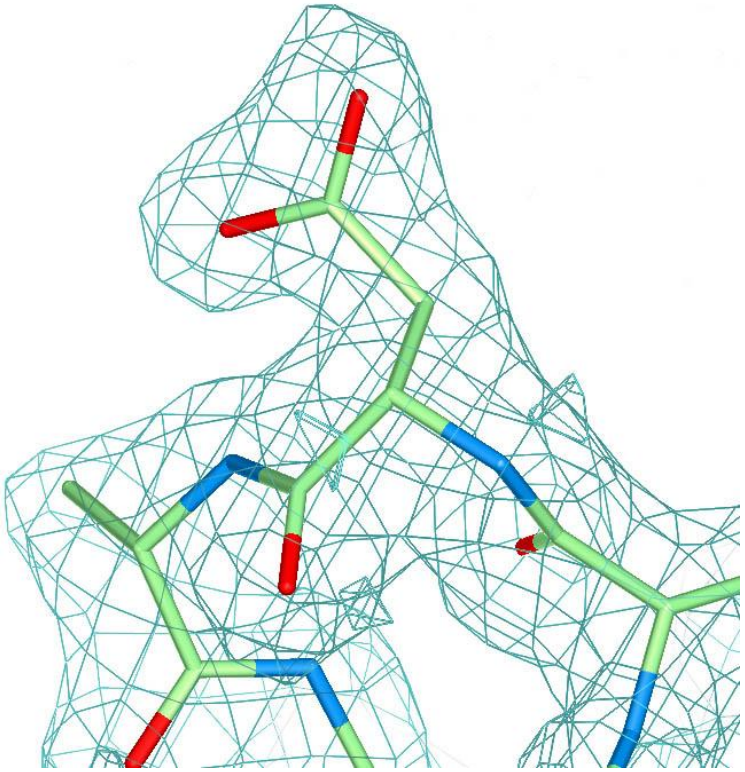


Control crystals **Growth in gel at 10 Tesla**

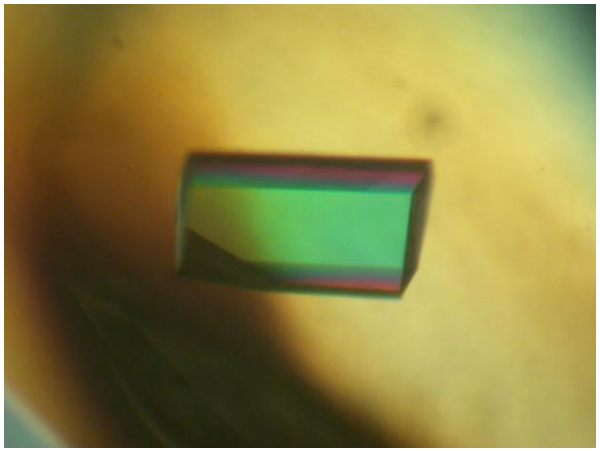
Reference: A. Moreno et al., "Protein crystal growth in gels and stationary magnetic fields". Crystal Research and Technology **42** (2007) 231-236

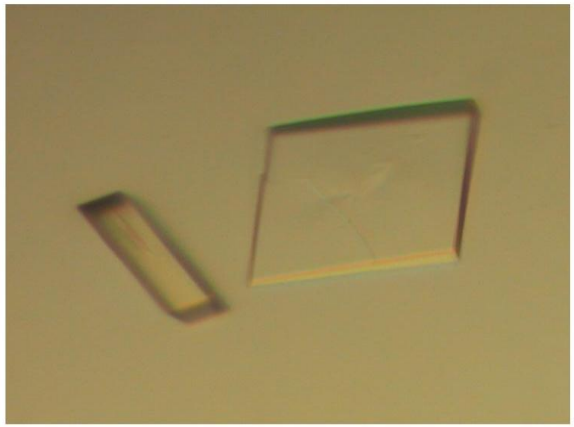


Electron density maps for thaumatin around the Asp 25 grown in magnetic field of 10 Tesla. This diffracted 1.5A resolution

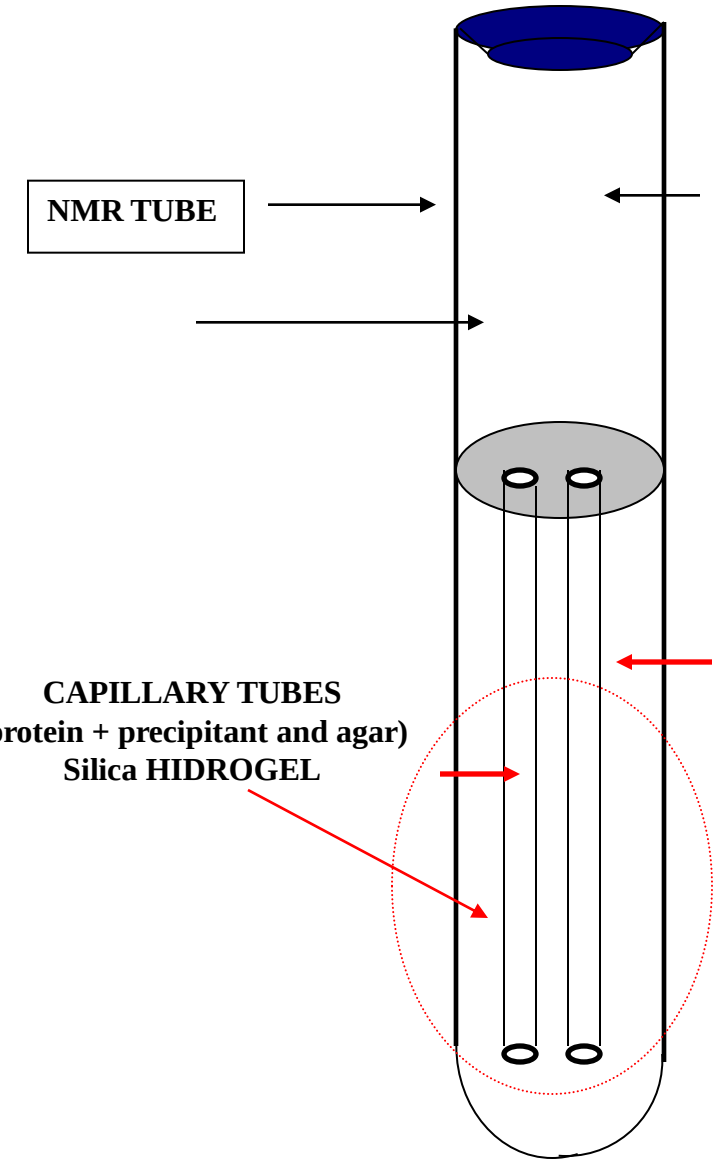


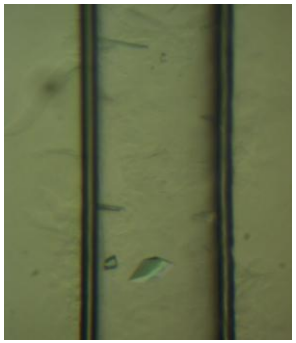
Application of this new method to the Aspartyl t-RNA synthetase from *Thermus thermophilus*





Application to the crystallisation of Aspartyl t-RNA Synthetase.





CONTROL

FIGURE 2a

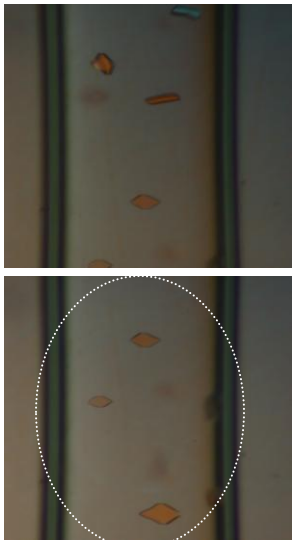
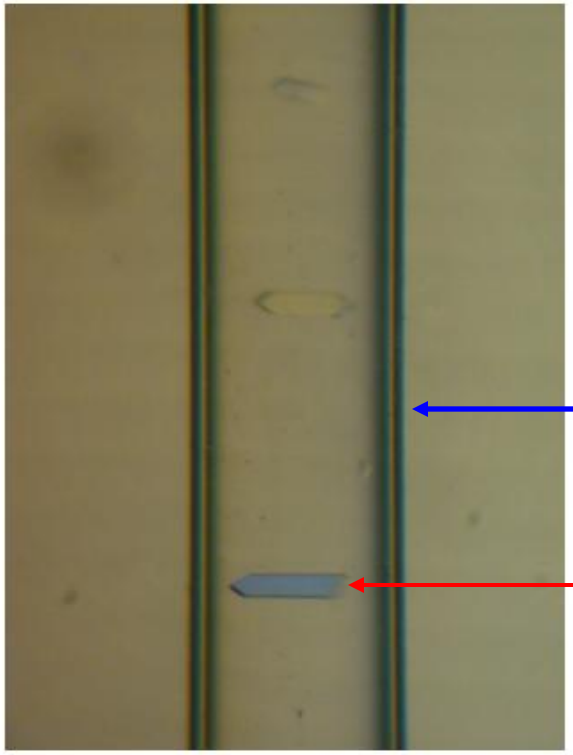


FIGURE 2b

MAGNETIC FIELD DIRECTION AND AREA OF MAXIMAL INTENSITY

The best results at best conditions for Aspartyl t-RNA Synthetase I

Direction of the magnetic field

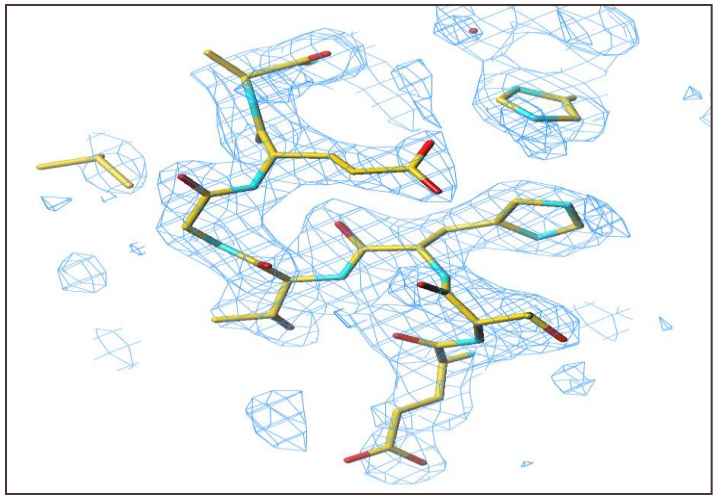


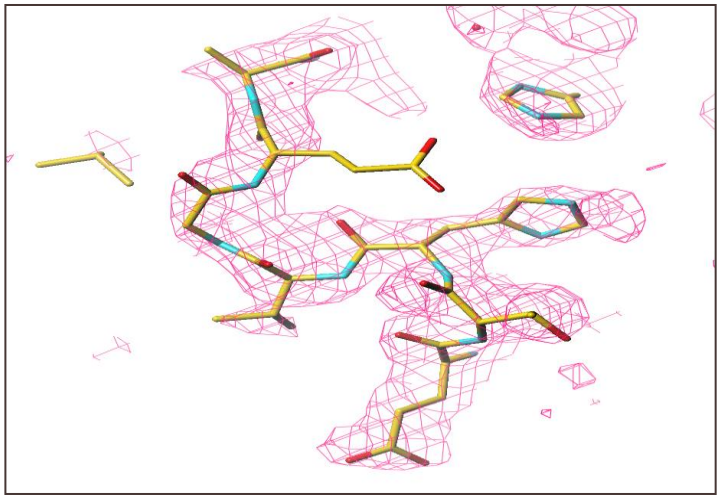
Capillary tube Ø=0.5 mm

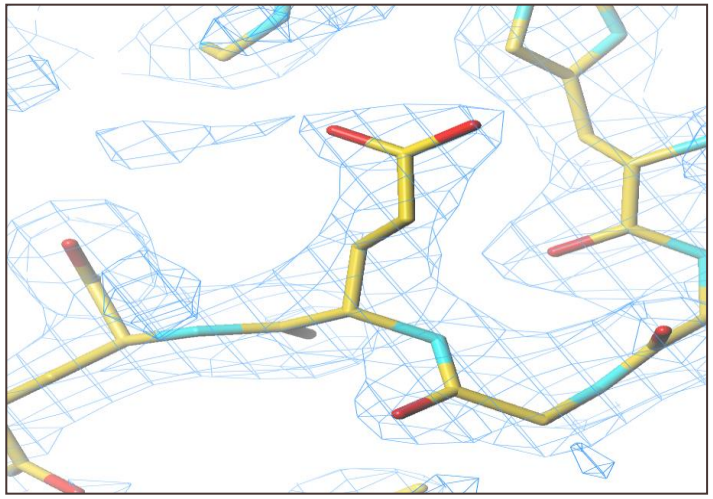
DRS-1 crystals

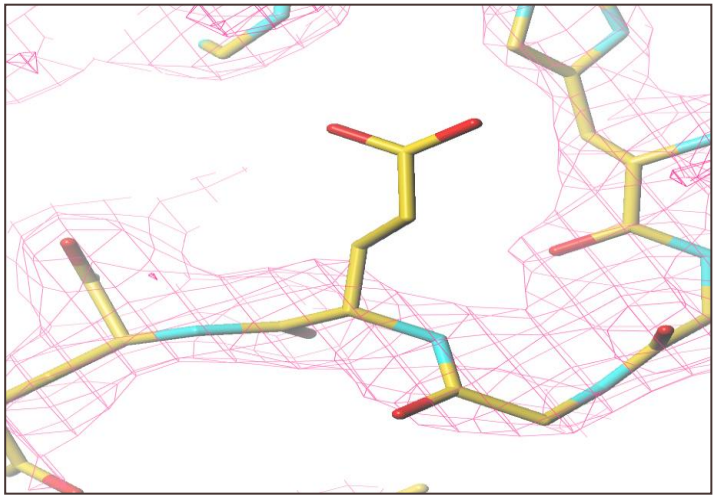
DRS-1 grown in gel/under magnetic field

DRS-1 grown in gel









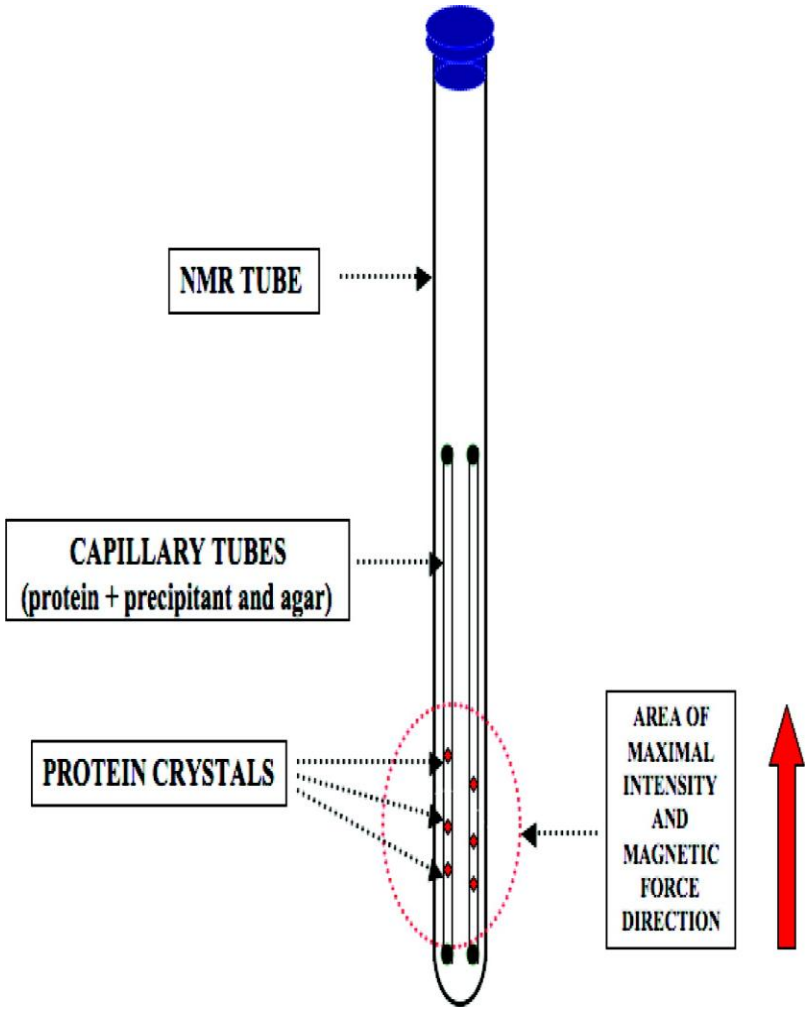
Crystal growth kinetics

Solution	Capillary Tube (Ø < 0.5 mm)	Gel	Gel/magnetic field (10 Tesla)
100-200 Å/s	10-30 Å/s	8-10Å/s	5-10 Å/s

The rate of crystal growth in microgravity is ranging from 5-8 Å/s.

So that, is microgravity necessary? At this stage maybe NOT

However… the most practical methodology to get high quality protein crystals is as follows…



Experimental set up to grow protein crystals in a NMR magnet of 11.75 T (500 MHz).

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Secondary structure content of the model proteins Thaumatin and Ferritin and the recombinant protein INHA-NAD.

Protein (Space group)	**Thaumatin** ($P4_12_12$)	**Ferritin** (F432)	**INHA-NAD** ($P6_122$)
Secondary structure composition	Helical 12 % Beta-sheet 36 %	Helical 73%	Helical 46% Beta-sheet 15%

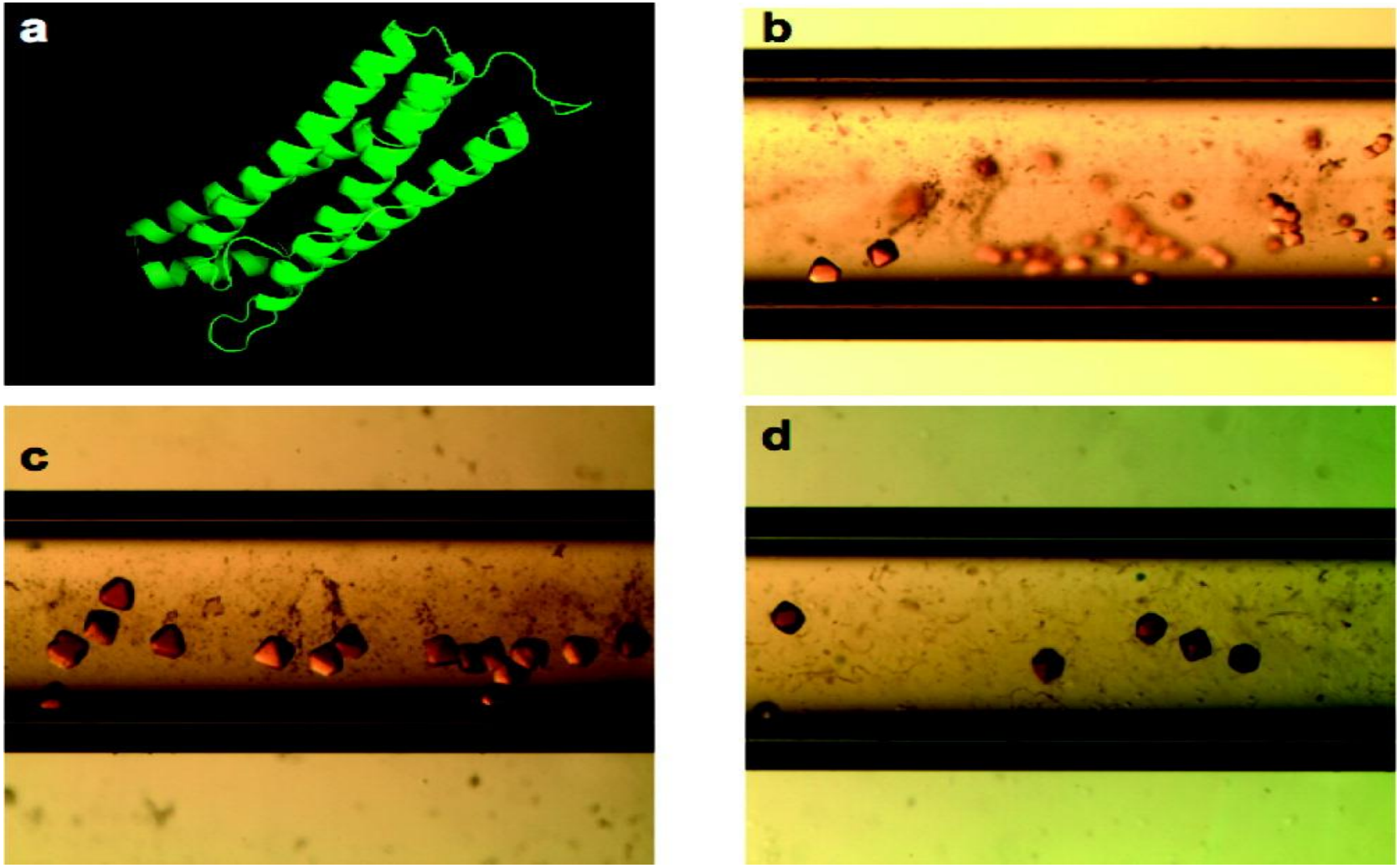
List of proteins and crystallographic space groups classified in terms of their polarity.

Protein	Lysozyme	Lysozyme	Thaumatin	Ferritin	INHA-NAD
Space group	$P4_32_12$	$P2_1$	$P4_12_12$	F432	$P6_122$
Polarity: (+) Polar (-) non-Polar	-	+	-	-	-

All space groups with a 2-fold axis perpendicular to the screw axis are not polar.

Table 1. The crystallization conditions for all proteins studied in this experiment.

Protein (mg/ml) (stock solutions)	**Lysozyme** (Sigma Cod. L-6876) (tetragonal) 120 mg/ml in 0.1M Na Acetate pH 4.6	**Lysozyme** (Sigma Cod. L-6876) (monoclinic) 40 mg/ml in 0.1M Na acetate pH 4.6	**Thaumatin** (Sigma Cod. T-7638) (tetragonal) 75 mg/ml in 0.1M Phosphate pH 7.0	**Ferritin** (Sigma Cod. F-4503) (cubic) 30 mg/ml in 0.1M Na Citrate pH 5.6	**INHA-NAD*** (wild-type) (hexagonal) 10 mg/ml (INHA was incubated for 1h with NADH (final 1mM)
Precipitant (stock solutions)	NaCl 120 mg/ml in 0.1M Na Acetate pH 4.6	$NaNO_3$ 0.6M in 0.1M Na Acetate pH 4.6	KNa Tartrate 45 % (w/v) in 0.1M buffer Phosphate pH 7.0	$CdSO_4$ 80 mM, Ammonium Sulphate 1.0M in 0.1M Na-Citrate pH 5.6	MPD 6-12% v/v in buffer Na Citrate 50 mM (pH 6.5), HEPES (100 mM pH 7)
Agar (stock solutions)	0.21% and 0.60% w/v	0.60% w/v	0.21% w/v and 0.60% w/v	0.21% w/v	0.21% w/v



Crystals of ferritin grown in the presence of a strong magnetic field of 11.75 T, (a) Ferritin PDB: pdblier (b) control: in solution, (c) solution in magnetic field, and (d) gel-growth (agar 0.07% w/v) in magnetic field. As a reference for the size of all crystals, the capillary tube is 1 mm in diameter.

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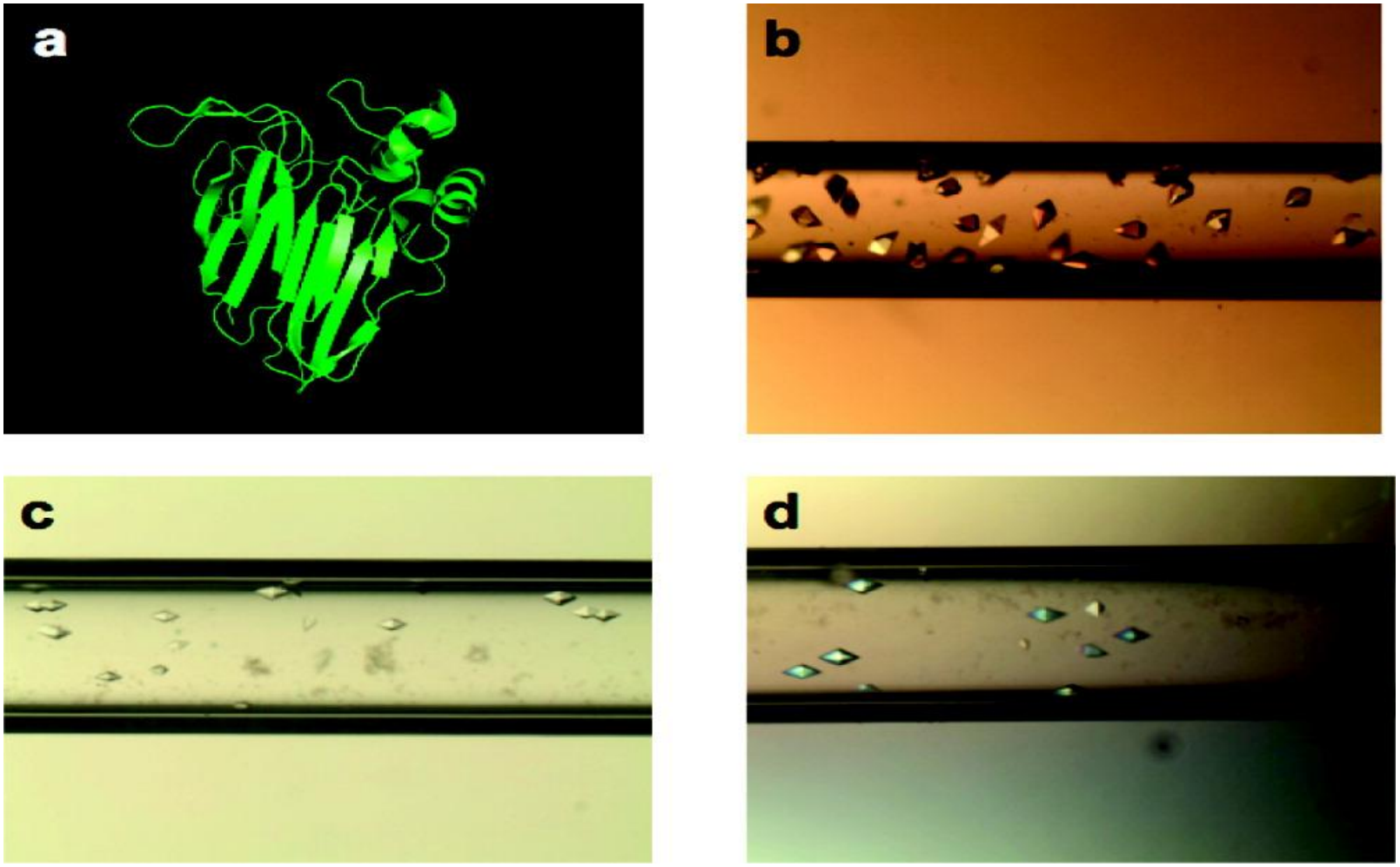

Crystals of thaumatin grown in the presence of a strong magnetic field of 11.75 T, (a) thaumatin PDB: 2Vl3 (b) control in solution, (c) solution in magnetic field, and (d) gel-grown (agar 0.07% w/v) in magnetic field. As a reference of the size of all crystals, the capillary tube is 1 mm in diameter.

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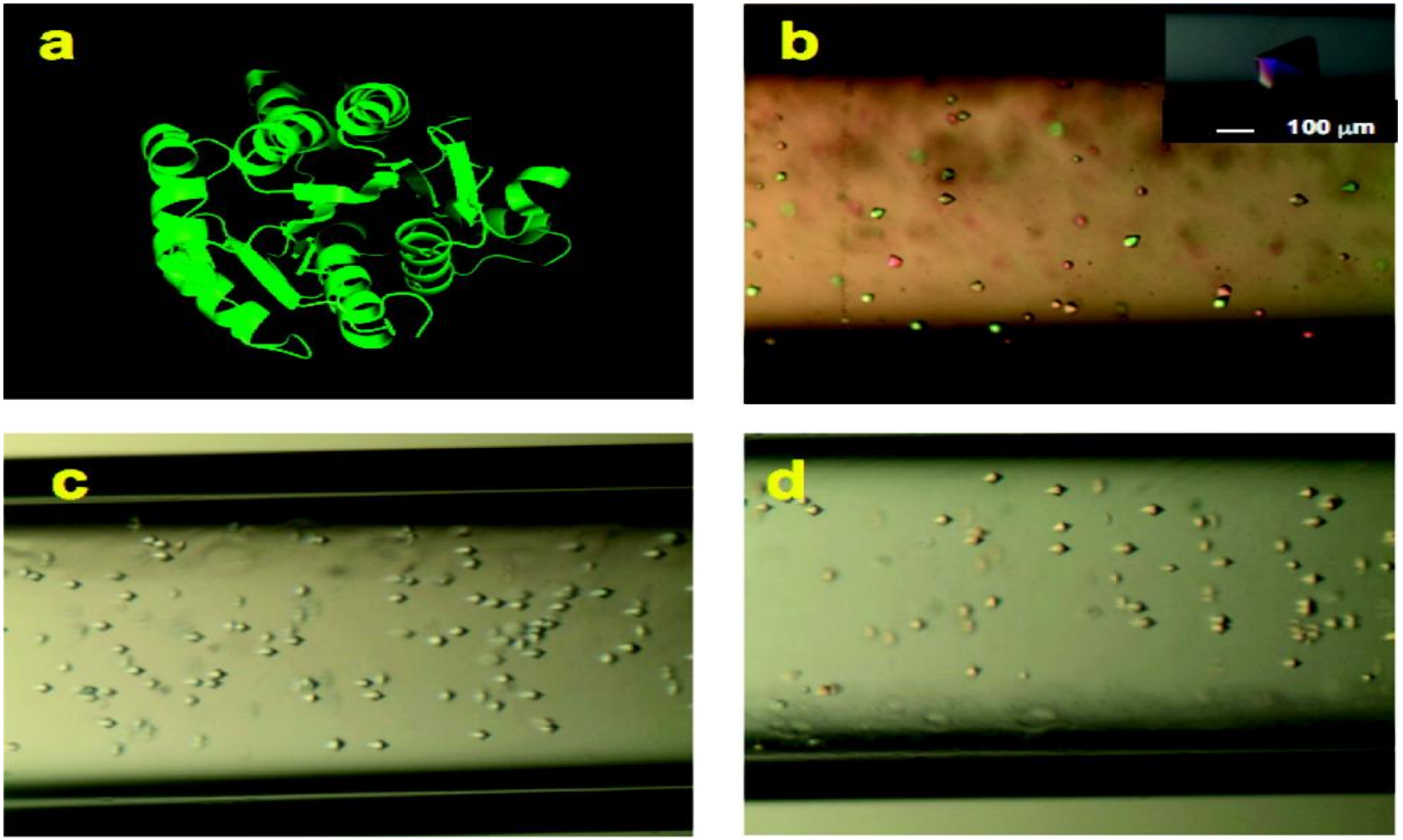

INHA-NAD crystals grown in a strong magnetic field of 11.75 T, (a) INHA-INH 3D-structure: INHA-NAD PDB file 2AQ8, (b) control (randomly oriented crystals): in-capillary tube, the inset shows a close up of the INHA-NAD crystal shape, (c) solution-growth of INHA-NAD in magnetic field, and (d) gel-growth (agar 0.07% w/v) of INHA-NAD in magnetic field. The size of the capillary tube is 1 mm in the inner diameter (b, c, and d).

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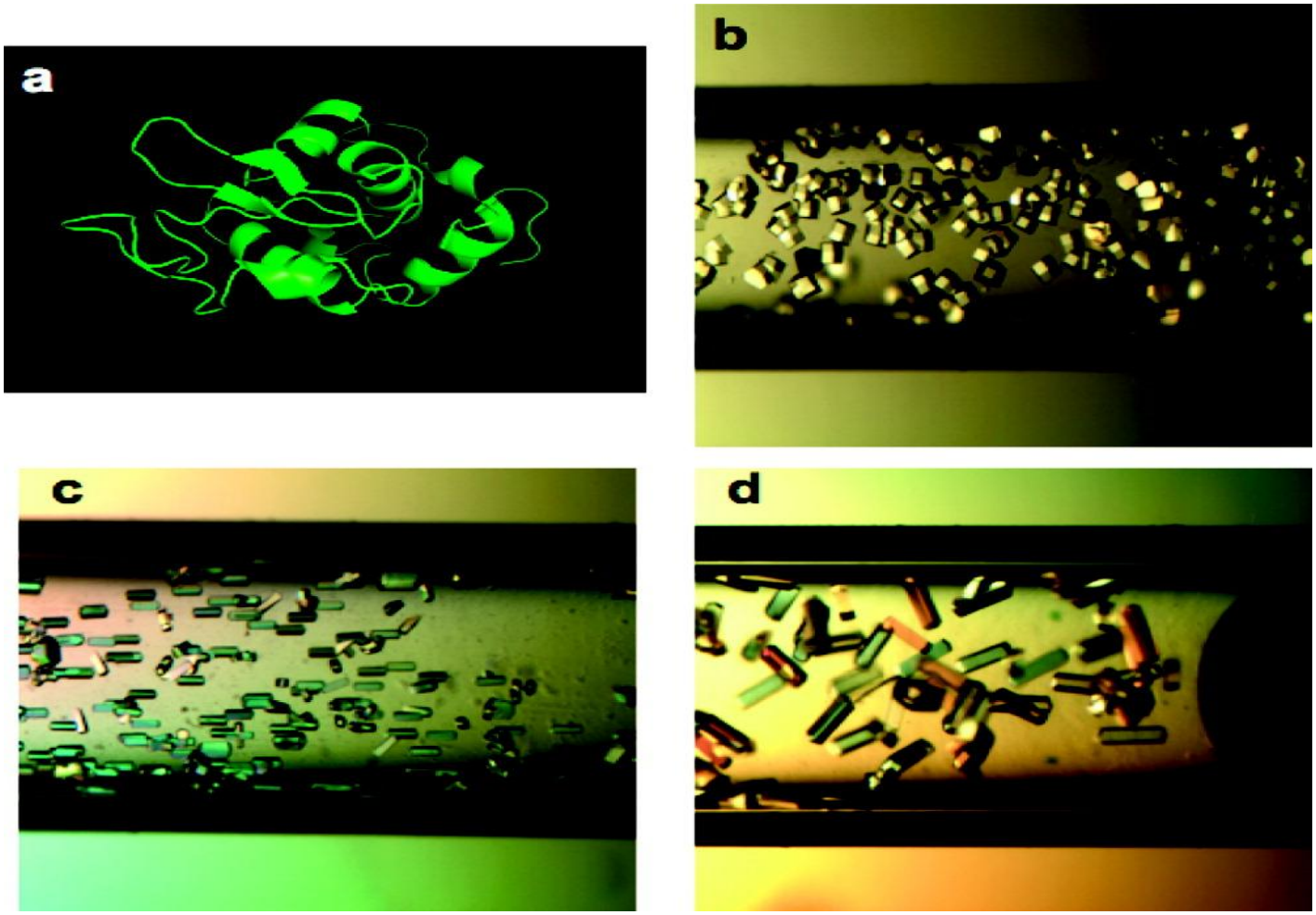
Table 3. Crystallographic X-ray data statistics for Ferritin crystals (F432, rich in α-helix), Thaumatin ($P4_12_12$, poor in α-helix contents).

	FERRITIN SOLUTION **(CONTROL)**	FERRITIN SOLUTION **MAGNETIC**	FERRITIN IN AGAR (0.07%) **MAGNETIC**	THAUMATIN SOLUTION **(CONTROL)**	THAUMATIN SOLUTION **MAGNETIC**	THAUMATIN IN AGAR (0.07%) **MAGNETIC**
Unit cell, a=b=c (Å)	182.42	182.28	182.65	57.92, 150.19	57.77 150.27	57.92 150.21
Resolution range (Å)	50-2.0 (2.05-2.0)	50 – 1.9 (1.94 – 1.90)	50 – 2.4 (2.46 - 2.40)	50-1.25 (1.28-1.25)	50 – 1.20 (1.23 – 1.20)	50 – 1.35 (1.38 – 1.35)
Rsymm (%)	7.5 (57.9)	6.1 (46.2)	9.8 (62.4)	4.8 (44.4)	5.4 (42.2)	5.7 (47.0)
Completeness (%)	99.7 (100)	99.6 (100)	99.5 (100)	82 (75.1)	99.4 (98.7)	99.8 (99.7)
Unique reflections	18,108	20,917	10,711	59,505	80,299	57,186
Average redundancy	11.4	15.2	15.3	5.6	8.7	8.7
Average intensity, $< I/\sigma(I)>$	12.0	15.1	9.3	12.3	11.6	11.9
% reflections with $<I/\sigma(I)> > 3$*	48.1	54.8	50.5	60.9	57.1	58.4
Wilson B-factor ($Å^2$)	25.7	23.4	34.2	10.0	9.4	11.2
Mosaicity (degrees)	0.58	0.53	0.68	0.35	0.29	0.28

*statistics for the highest resolution shell



Crystal of lysozyme grown in the presence of a strong magnetic field of 11.75 T, (a) lysozyme PDB: l93L (b) control in solution, (c) solution in magnetic field, and (d) gel-grown (agar 0.2% w/v) in magnetic field. As a reference of the size of all crystals, the capillary tube is 1 mm in diameter.

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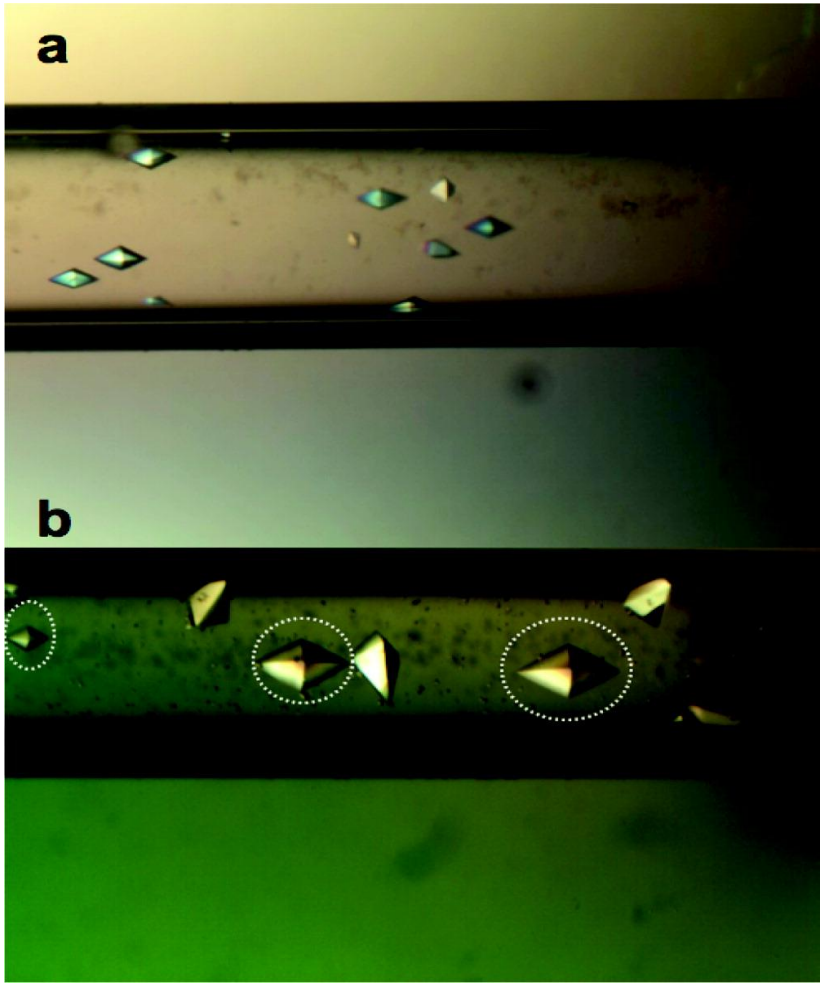

Thaumatin crystals grown in a strong magnetic field of 11.75 T at two concentrations of agar: (a) 0.07% and (b) 0.2% w/v. The three dotted circles show the oriented crystals along their *c*-axis in the direction of the magnetic field. As a reference of the crystal size, the capillary tube is 1 mm in diameter in both cases.

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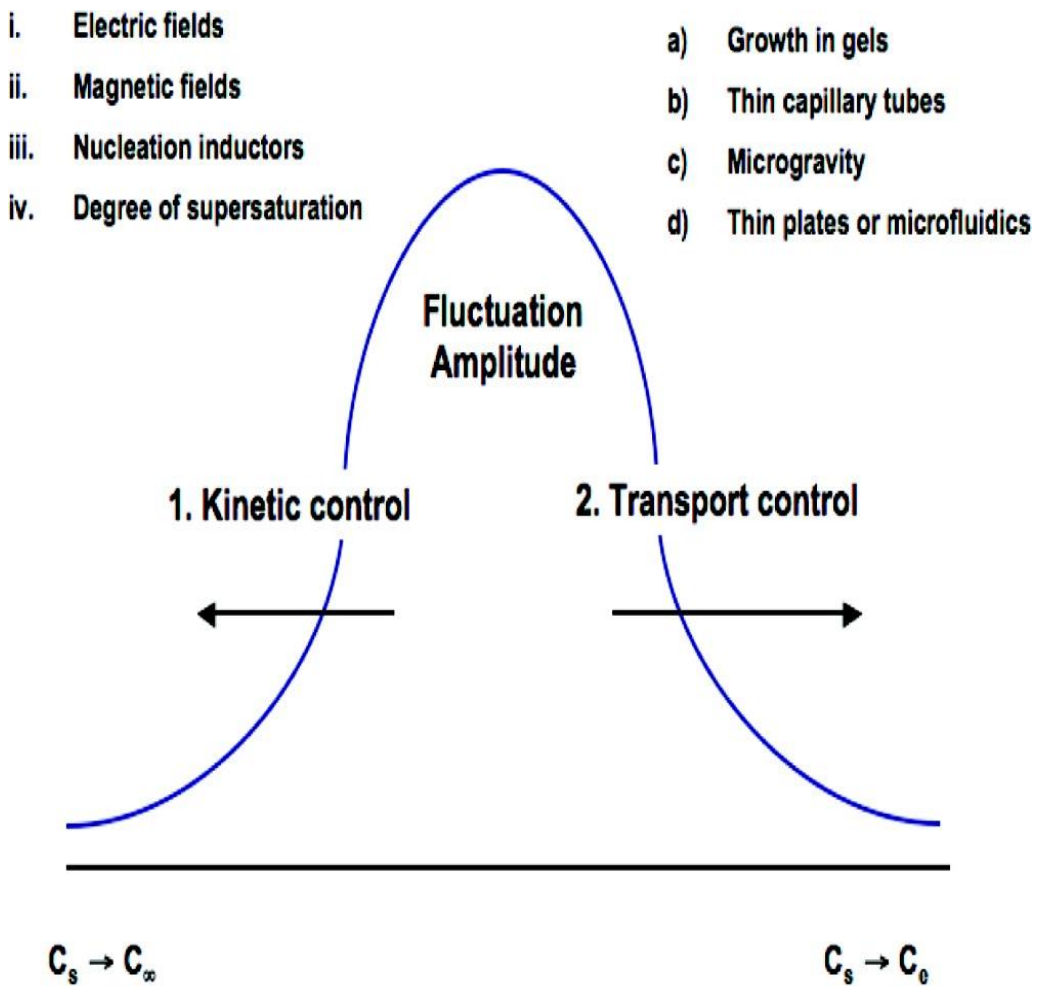
Table 4. Crystallographic X-ray data statistics for Lysozyme crystals (Space group: $P4_32_12$) and Thaumatin (Space group: $P4_12_12$) grown in solution and in highly concentrated agar in a strong magnetic field of 11.75 T.

	LYSOZYME SOLUTION **MAGNETIC**	LYSOZYME AGAR (0.2%) **MAGNETIC**	THAUMATIN IN AGAR (0.07%) **MAGNETIC**	THAUMATIN AGAR (0.2%) **MAGNETIC**
Unit cell, a=b, c (Å)	78.58 36.89	79.04 36.99	57.92 150.21	57.68 149.63
Resolution range (Å)	50 – 1.2 (1.23 – 1.20)	50 – 1.20 (1.23 – 1.20)	50 – 1.35 (1.38 – 1.35)	100-1.30 (1.33 - 1.30)
Rsymm (%)	4.2 (20.1)	4.6 (17.8)	5.7 (47.0)	7.5 (45.5)
Completeness (%)	99.2 (100)	99.0 (100)	99.8 (99.7)	91.2 (95.9)
Unique reflections	36,418	36,814	57,186	57,927
Average redundancy	8.7	8.4	8.7	6.6
Average intensity, $< I/\sigma(I)>$	17.0	15.6	11.9	9.3
% reflections with $<I/\sigma(I)> > 3$*	75.4	77.5	58.4	51.1
Wilson B-factor ($Å^2$)	9.7	8.6	11.2	10.2
Mosaicity (degrees)	0.29	0.14	0.28	0.24

* statistics for the highest resolution shell.



Rate versus transport control or kinetic control of the crystal growth process (adapted from Vekilov et al.).(32) The right-side of the figure shows different methods for increasing the crystal quality by transport control, while the left-side lists some of the approaches that can influence or affect the kinetics of the process.

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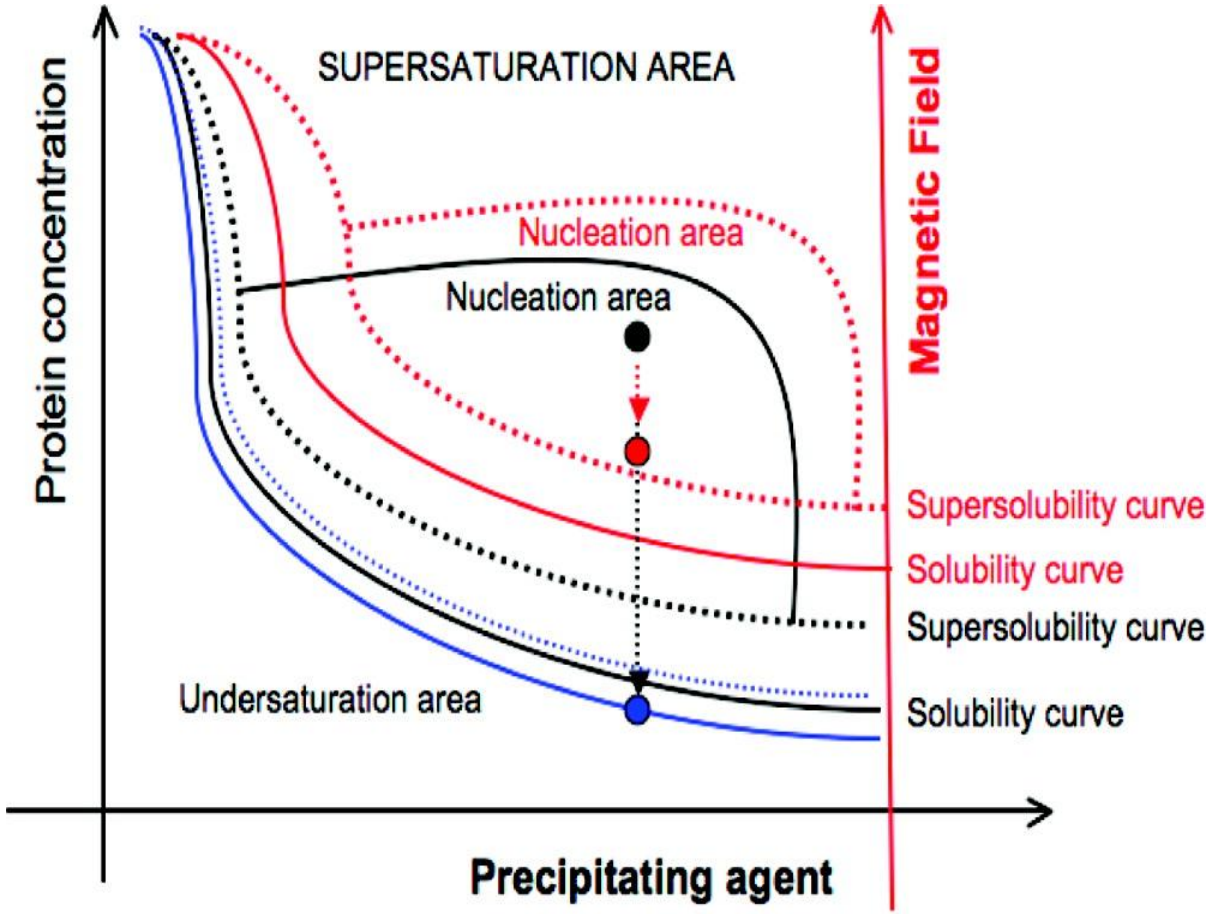

Conceptual protein solubility plots of batch crystallization under different experimental conditions. The black line represents the control solubility curve, the red line is the solubility curve in the presence of the magnetic field, and the blue line is the resultant curve after the experiment. The black, red, and blue spots represent the fixed crystallization conditions (protein concentration and precipitating agent ratio) for batch crystallization at the beginning, under the strong magnetic field influence, and at the end of the experiment, respectively.

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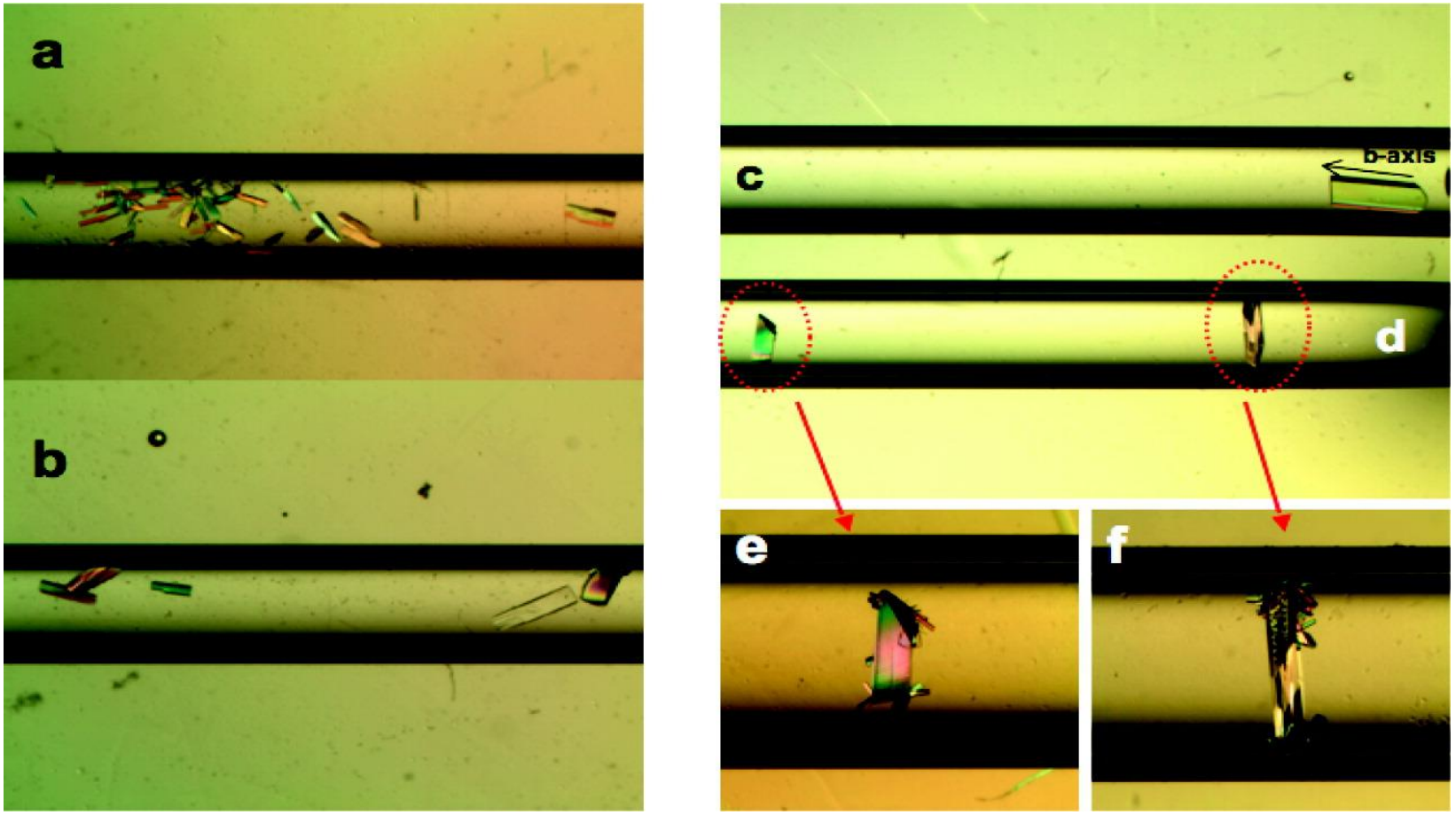
Crystal growth kinetics

Solution	Capillary Tube (Ø < 0.5 mm)	Gel	Gel/magnetic field (11.75 Tesla)
100-200 Å/s	10-30 Å/s	8-10Å/s	5-10 Å/s

The rate of crystal growth in microgravity is ranging from 5-8 Å/s.

So that, is microgravity necessary? At this stage maybe NOT



The capillary tube shows (a) the monoclinic lysozyme crystals (*P*21) grown in solution, (b) monoclinic crystals grown in solution and in magnetic field after 96 h out of the magnetic field, (c) monoclinic crystal grown in agar 0.2% (w/v). The lower capillary tube (d) shows the different shape of the monoclinic lysozyme crystals grown in agar (0.2% w/v) inside a magnetic field of 11.75 T for 2 days. As the figure shows, two crystals appear properly oriented. After 96 h out of the magnetic field, the same crystals grown in gel from Figure 9d are shown (insets e and f), but some satellite crystals (having a monoclinic shape) were starting to form in both crystals.

Published in: Sachin Surade; Takashi Ochi; Daniel Nietlispach; Dima Chirgadze; Abel Moreno; *Crystal Growth & Design* **2010,** 10, 691-699.

Conclusions

- The magnetic fields are a very promising tools for controlling the number of crystals, crystal orientation, and high quality.
- The magnetic field coupled to the crystal growth in gels is a very promising method for the enhancement of the crystal quality for X-ray Crystallography. The kinetics and transport phenomena of crystal growth can be controlled at the same time.
- The 3D structure is improved when magnetic field was applied. So that, the main advantage of this method is the increase of the size, the crystal quality and the obtaining of crystals in a shorter time.
- We must consider that macromolecular impurities play an important role along the crystal obtaining as well as the crystal growth method for high resolution X-ray crystallography research.
- Finally, this method is not applicable when you do not have the batch crystallisation conditions or your protein takes longer time to be crystallised. The use for a magnet 500 MHz is so expensive.

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