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Among various methods for structural studies of biological macromolecules, neutron scattering and diffraction have a unique feature that the contrast between the scattering length density of the molecules and that of the solvent can be varied easily by changing D<sub>2</sub>O content in the solvent. This “contrast variation” technique enables it to obtain information on internal fluctuations or a variation of scattering length density of the molecules of interest. Here, in order to explore the possibilities of neutron fiber diffraction, the contrast variation technique was applied to measurements of neutron fiber diffraction of muscles. The neutron fiber diffraction patterns of frog sartorius muscles were measured under the relaxed state where no tension of the muscle is produced, and under the rigor state where the myosin heads of the thick filaments bind tightly to actin in the thin filaments, in various D<sub>2</sub>O concentrations. It was shown that under both states, there were reflections having distinct contrast matching points, indicating a variation in the scattering length density distribution in the unit cell of the muscle structure. Analysis of the equatorial reflections showed that the phase information of these reflections is obtained, that the density projected to a plane perpendicular to the axis of the muscle is different between the thick filament region and the thin filament region, and that the projected density of the thick filament changes as the state of the muscle changes from the relaxed state to the rigor state. Analysis of the meridional reflections of the thick filament suggested that in addition to contributions from the myosin head regions, the backbone region of the thick filaments contributes to the intensity of the meridional reflections as well.

Keywords: fibre diffraction, muscle, neutron diffraction

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#### Molecular orientation of a collagen hydrogel with high mechanical strength

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Collagen is a major fibrous protein of the extracellular matrix. The molecule has a triple-helical structure and is known to assemble to form fibrils. Furthermore, collagen fibrils organized into one direction to form fibers in tendons and ligaments. The orientation of these molecules is known to be a significant factor in the mechanical strength of natural tissues such as bone, tendon, ligament and the cornea. Accordingly, the construction of collagen gels with oriented fibrils attracts much attention for tissue engineering. We reported a novel and simple method to prepare collagen gels with molecularly oriented fibrils. In this study, x-ray diffraction measurements

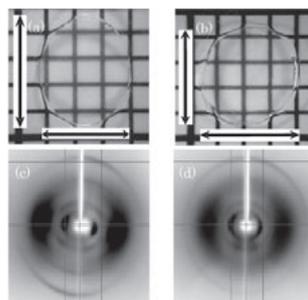


Fig. 1 Optical photomicrographs and X-ray diffraction patterns of oriented (a and c) and non-oriented (b and d) collagen gels, respectively.

were performed to confirm the molecular orientation in the gels. Amazingly, the pattern of oriented gel was quite similar to that of native tendon collagen, which shows narrower arc pattern than that of non-oriented gel (Fig.1). The results of the diffraction patterns clearly suggested a highly-ordered molecular orientation of the fibrils in the oriented gels. We succeeded in the controlling of the orientation of the fibrils in the gel. This technique is considerably effective as the regenerative medicine technology.

Keywords: collagen, fibre diffraction, molecular orientation

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#### Multiple scattering of light by collagen nanofibres in biological tissues

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Some biological tissues look like a fibrillar texture, which can provide an intense response to incident electromagnetic waves. This gives the possibility to perform investigations of optical properties and structure of such natural textures. Natural collagen fibrils are encountered, for example, in the cornea and sclera. Both cornea and sclera tissues are essentially binary nano-composite materials, consisting of collagen fibrils embedded in a water-based mucopolysaccharide background substance, whose refractive index is different from the refractive index of collagen fibres. It is well known (e.g. see [1]) that difference in the structure of cornea and sclera is governed by the arrangement and sizes of collagen fibrils in the background substance, which makes such a difference in the optical performance of the transparent cornea and the opaque sclera. Here we explain optical properties of cornea and sclera by 2D quasi-crystalline lattice model constructed from rods (fibres) of dielectric constant infinite in one direction. Bloch proved in 1928 that waves in periodic media can propagate without scattering, their behaviour governed by a periodic envelope function multiplied by a plane wave [2]. This technique can be applied to electromagnetism by considering Maxwell's equations as an eigenvalues and eigenfunctions problem in analogue with Schrödinger's equation (e.g. see [3, 4]). Such approach to considered model of quasi-periodic fibrillar texture takes into account a multiple scattering of light by collagen fibres.

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Keywords: fibre diffraction theory, electromagnetic wave theory, quasicrystal scattering

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#### Crystal structures of chitosan and its complexes with hydrogen halides

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In order to elucidate the intermolecular interactions involved in complex formation of chitosan with hydrogen halides, crystal structures of chitosan complexes with HBr and HI were analyzed based on synchrotron X-ray fiber diffraction data (BL40B2, SPring-8, Hyogo, Japan). Both crystals are isomorphous and belong to the monoclinic space group  $P2_1$ . The unit cell constants are  $a = 9.299(9)$ ,  $b = 9.504(8)$ ,  $c$  (fiber axis) =  $10.41(1)$  Å and  $\beta = 106.93(8)$  deg, and  $a = 9.46(2)$ ,  $b = 9.79(2)$ ,  $c$  (fiber axis) =  $10.33(2)$  Å and  $\beta = 105.1(2)$  deg for HBr and HI complexes, respectively. The final packing models were obtained by the linked-atom least-squares refinement, which gave R-factors of 0.192 for HBr complex (93 observed spots) and 0.193 for HI complex (44 observed spots). The halide ions are aligned along the c-axis at intervals of about 5 Å and are surrounded by four polymer chains. In an asymmetric unit, there are two halide ions. One ion accepts three hydrogen bonds from  $\text{NH}_3^+$  groups (N2 nitrogen). The other one participates in one hydrogen bond from N2 and two hydrogen bonds from primary hydroxyl groups (O6 oxygen). In addition, the chitosan chains are linked by N-H...O and C-H...O hydrogen bonds along the b-axis direction. The crystal structure of the hydrated form of chitosan was reanalyzed using synchrotron X-ray fiber diffraction data (BL38B1, SPring-8). The chitosan chains make sheet structures parallel to the bc-plane and these sheets stack along the a-axis. Water molecules form columns between these sheets. The sites of the halide ions in the complex crystals are similar to those of the water molecules in the hydrated form. It was suggested that the columnar structure of water in the hydrated form plays an important role for the complex formation.

Keywords: complexes, fibre diffraction, synchrotron radiation

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#### Structural analysis of F-actin using fiber diffraction

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Actin is one of the most abundant proteins and works in the eukaryotic cells. Actin has two states – monomeric G-actin and polymerized F-actin. The F-actin is a functional form in muscle and cellular transportation. Also the processes of polymerization of actin are important for cell motility. In 1990, crystal structure of actin-DNase I complex was solved by Holmes group. Since then, a lot of crystal structures of actin were solved. Actin has a nucleotide-binding cleft enclosed by two major domains. In almost all of actin crystal structures, the cleft is closed and the two domains are tilted each other in the propeller-like manner. The conformation appears to be typical G-actin conformation. In the back-to-back paper, his group also proposed the structural model for F-actin using the fiber diffractions from well-oriented sols. The monomer arrangement and orientation in the F-actin model have widely been accepted. However, to precede the next steps of actin studies, for example detailed interaction between F-actin and myosin, actin ATPase and polymerization, we need a precise model for the F-actin structure. We recorded the diffraction patterns from well-oriented F-actin sols at SPring-8. Using the diffraction pattern up to 3.3 Å resolution in the radial direction and 5.5 Å along the equator, we made a new model for F-actin structure. We put crystal structures in helical arrangement of F-actin and fitted the model to the reflection data using a combination of

normal mode motions of G-actin. After that, we refined the structure using the MD refinement by FX-plor. We found a novel conformation of actin in F-actin filament. In this conformation, whole molecule is flat and the nucleotide binding cleft remains closed.

Keywords: actin, fiber diffraction, macromolecular polymer

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#### Computational methods in fibre diffraction

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Fibre diffraction is used in structure determination of filamentous biological polymers when conventional macromolecular crystallography cannot be applied. The technique made a critical impact in the early days of structural biology, but since then has gone largely unheralded, partially owing to the limitations of available computational tools. With the advancement of computer and synchrotron technologies and the arrival of new structural problems, creating reliable software for fibre diffraction research became both feasible and urgent. In collaboration with fibre diffraction communities in North America and Europe, we have developed and integrated a collection of such software tools. These tools include WCEN for analysis of diffraction patterns, correcting systematic effects and mapping diffraction data into reciprocal space, RAD for angular deconvolution of diffraction data from partially oriented specimens, FX-PLOR for model building and refinement against high resolution diffraction data, and FNV for analysis using Fourier-Bessel transforms and syntheses. The software collection, already successfully applied in the study of non-crystalline filamentous systems such as amyloids and viruses, together with the CCP13 software suite, covers all aspects of fibre diffraction. The work is supported by FiberNet, a Research Coordination Network for Fiber Diffraction from Biological Polymers and Assemblies established through NSF grant MCB-0234001.

Keywords: fibre diffraction, macromolecular structure determination, computational method

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#### Application of neutron imaging plate system to crystal structure analysis of deuterated polymers

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The 2-dimensional neutron imaging plate system BIX-3, developed for the structural biology, has been successfully applied to the crystal structure analysis of various kinds of synthetic polymers, and the extraction of hydrogen atom positions has been made with high accuracy. The polymers investigated here were polyethylene, polyoxymethylene, isotactic polypropylene, poly(ethylene oxide),