CO1-1

Small-angle X-ray scattering analysis of stabilized di-nucleosomes

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INTRODUCTION: In eukaryotic cells, genomic DNA is packaged into chromatin in the nucleus. The nucleosome is the repeating unit of chromatin, composed of 145-147 base pairs of DNA wrapped around a histone octamer containing two molecules each of histone proteins H2A, H2B, H3, and H4. Chromatin structure changes dynamically during various nuclear processes, such as gene transcription, DNA repair, and replication. These structural changes are influenced by histone modifications and chromatin-associated factors. Because the arrangement of neighboring nucleosomes is an important determinant of chromatin conformation, di-nucleosomes provide a simple model system for structural analysis. We analyzed di-nucleosomes by small-angle X-ray scattering (SAXS). To develop methods for structural analysis of chromatin conformation in solution using a di-nucleosome system, we examined a sample stabilization strategy to improve homogeneity for SAXS measurements.

EXPERIMENTS: Di-nucleosomes were assembled by in vitro reconstitution. Recombinant histones were combined with a DNA fragment under high-salt conditions, followed by a gradual decrease in salt concentration to promote nucleosome formation. The assembled di-nucleosomes were subsequently isolated by polyacrylamide gel electrophoresis [1]. Di-nucleosomes were gently fixed in a continuous sucrose gradient containing glutaraldehyde (GraFix) [2]. The samples were then dialyzed against SAXS buffer and concentrated prior to small-angle X-ray scattering (SAXS) measurements. SAXS intensity of the corresponding buffer was measured for background subtraction using the same procedure as for the samples. The scattering data were collected using a laboratory-based SAXS instrument equipped with a high-brilliance point-focused Cu Kα source. To evaluate sample quality, including aggregation and dissociation, sedimentation velocity experiments were performed by analytical ultracentrifugation (AUC) [3]. In addition, the oligomeric state of the samples was analyzed by mass photometry.

RESULTS: At a concentration of 0.8 mg/mL, the SAXS sample showed substantial polydispersity. The AUC profile indicated the presence of 2- and 3-mer associated species, aggregated particles, and degraded components in addition to the di-nucleosome fraction, with degraded species accounting for approximately 50% of the total. Mass photometry yielded results consistent with the AUC analysis, despite the dilution required for the measurement. Based on these results, we used mass photometry to examine the GraFix fractions, dialysis and sample concentration conditions, and identified conditions that reduced sample heterogeneity. These analyses will be further pursued to establish sample preparation conditions for more homogeneous SAXS measurements of di-nucleosomes in solution, providing a basis for future structural studies of chromatin assemblies with additional components.

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CO1-2

Development of a full circumference ellipsoidal neutron supermirror

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INTRODUCTION: A new research reactor construction project, of which Kyoto University is one of three core institutions, is underway at the Monju site in Tsuruga City, Fukui Prefecture. The reactor's primary purpose is to produce results for academic and industrial applications using neutron beams and contribute to various fields, especially the local community. Effectively using the focusing mirror is one of essential to competing with leading institutes in the fields of neutron beam science and technology. We have developed a fabrication method for aspherical focusing supermirrors with a metal substrate [1]. The metal substrate is robust and ductile, and it can produce a steeply curved surface with high shape accuracy. It can also withstand high radiation and temperatures, even near the neutron target and moderator. We have achieved a smooth surface for high-m supermirror coating. Here, m is the maximum critical angle of the mirror in units of the critical angle of natural nickel. In this report, we present our progress on high-m neutron-focusing supermirrors on a full circumference ellipsoidal metal substrate.

EXPERIMENTS: We fabricated ellipsoidal metallic substrates with electroless nickel-phosphorus plating using ultra-high-precision cutting technology, correction processing, and mechanical precision polishing. Initial precise manufacturing was conducted on a CNC machine in the KURNS workshop for developing neutron optical devices. The ultra-precise manufacturing, polishing, and cleaning of the substrates were performed at RIKEN. Supermirror coating was performed using an ion beam sputtering machine (KUR-IBS) at KURNS. m=6 NiC/Ti supermirrors, of which number of maximum effective number of layer 12180, were deposited on a metal substrate with the semi-major and semi-minor axes of the supermirror are 1,250 mm and 65.4 mm, respectively. The full-circumference ellipsoidal mirror consists of 54 substrates, which were assembled at RIKEN to form one ellipsoidal focusing mirror. We also evaluated the neutron reflectivity of supermirrors deposited on a silicon (Si) substrate that were deposited simultaneously with the metal substrates at the CN-3 port of the KURLS reactor and the C3-1-2(MINE) port of the JRR-3 reactor.

RESULTS: As shown in Fig.1, a full circumference ellipsoidal m=6 supermirror with a length of 0.9 m was completed and installed to J-PARC MLF BL06(VIN ROSE). Although these are still preliminary results, we obtained a focusing spot as designed.

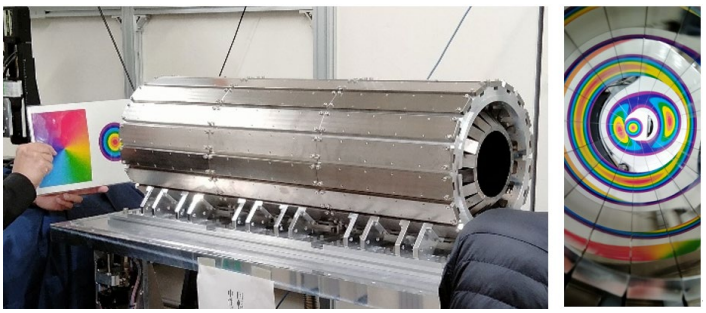

Fig.1 Photograph of a full circumference, ellipsoidal neutron supermirror made of 54 mirror pieces.

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[2] M. Hino, *et al.*, Nucl. Instr. and Meth., **797**, 265 (2015)

CO1-3

Characterization of cold-neutron flux of the TRIUMF Ultracold Advanced Neutron Source

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INTRODUCTION: The TRIUMF Ultracold Advanced Neutron (TUCAN) collaboration has been developing an accelerator-driven superthermal ultracold neutron (UCN) source at TRIUMF [1]. The method adopted here utilizes inelastic scattering of neutrons in superfluid helium, which exhibits a strong energy dependence around 1 meV. Characterization of the cold neutron spectrum incident on the superthermal converter is therefore a critical aspect of this source. To carry out this characterization effectively, we developed a neutron activation analysis method using cadmium and borosilicate glass as filters, sensitive to thermal and cold neutron energies, respectively. This method was tested at KUR-CN3 beamline and then used at TRIUMF to characterize the neutron spectra of the TUCAN source with different operational conditions.

EXPERIMENT In each data-taking run, a gold foil (typically 20 mm×20 mm× 50 μm) was placed in a holder, as shown in Fig. 1, with different filters positioned in front of it. The proton beamline typically operated for 5 minutes. The neutron flux was subsequently evaluated by counting the characteristic gamma rays at 412 keV emitted following neutron capture on ^{197}Au.

RESULTS: The results of the activation measurements for different moderator conditions of the TUCAN source are shown in Fig 2. The two different moderator conditions measured were 1) with room temperature heavy water (D_2O) moderator, and 2) with D_2O and liquid deuterium (LD_2) moderator. The horizontal axis is the thickness of the borosilicate glass plates used as filters. The observed difference in slopes between the two conditions indicates a change in the neutron temperature between these configurations. A more detailed analysis is currently underway.

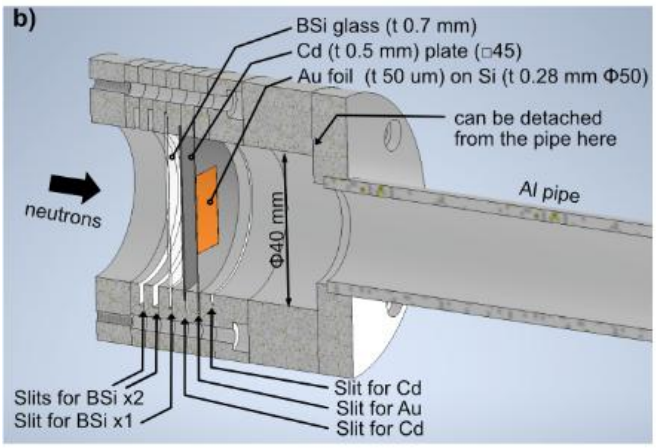

Fig. 1. Holder used to install gold foils and filters in the cold neutron monitoring port of the UCN source.

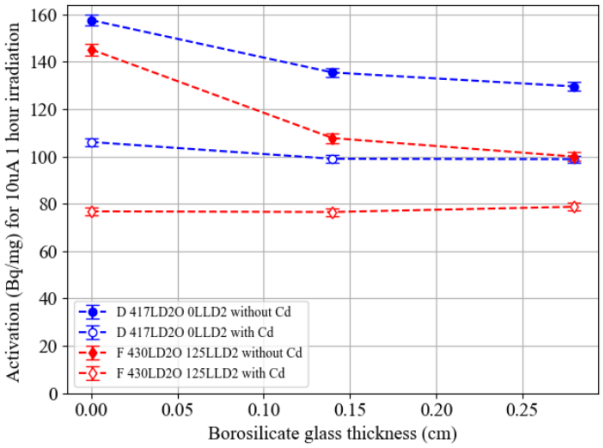

Fig. 2. Results of the activation measurement with different moderator conditions of the UCN source

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CO1-4

Development of absorption grating devices for CN-3 beamline at KURNS

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INTRODUCTION: Neutron imaging techniques are powerful, versatile, nondestructive analytical tools used in many research fields. Since its proposal in 2006[1], neutron Talbot-Lau interferometry (nTLI) has attracted considerable attention; it uses three gratings: a source grating (G0), a beam splitter grating (G1), and an analyzer grating (G2). G0 and G2 are absorption gratings, while G1 is a phase grating. Absorption gratings are important devices, and gadolinium (Gd) is used in their fabrication due to its high absorption cross section. This study presents a novel fabrication method for absorption gratings that uses an optimized Gd-based multilayer for ultra-high precision cutting.

EXPERIMENTS:
The Gd-based multilayer consists of gadolinium (Gd) and titanium (Ti). The G0 and G2 gratings were created by microcutting the multilayer on a substrate. These gratings were evaluated using an nTLI installed in the CN-3 port of the Kyoto University Reactor (KUR) [2]. For the nTLI at the CN-3 port, which detects thermal neutrons, the required effective Gd thickness is greater than 20 μm. To achieve this thickness, the Gd film was coated using an ion beam sputtering instrument (KUR-IBS) at KURNS, as shown in Fig. 1.
We fabricated a very flat aluminum substrate with a thickness of 20 mm for ultra-high precision cutting prior to deposition. We used the Gd/Ti bilayer to fabricate the G0 and G2 gratings, which had 2,000 and 400 layers, respectively. The deposition times for the Gd and Ti layers were five and 30 seconds, respectively. After sputtering, we shaped the Gd/Ti multilayer using an ultra-high-precision cutting machine (NPIC-M200, Nagase Integrex Co., Ltd.) at RIKEN. The G0 and G2 grating pitches were 125 μm and 8.5 μm, respectively, within 50 mm squares.

Fig.1 Typical measured visibility of the nTLI installed to the CN-3 beamline.

RESULTS: Fig.2 shows the measured visibility of the newly fabricated G0 and G2 gratings at the nTLI at the CN-3 port. The average visibility of the pixels in the central area (marked by the yellow rectangle) was 0.28. Based on neutron transmission efficiency, we estimated the total thicknesses of the G0 and G2 films to be 46 μm and 8.5 μm, respectively. Considering the lesser thickness of the G2 grating for the neutron wavelength of the CN-3 beamline, the average visibility is reasonable.

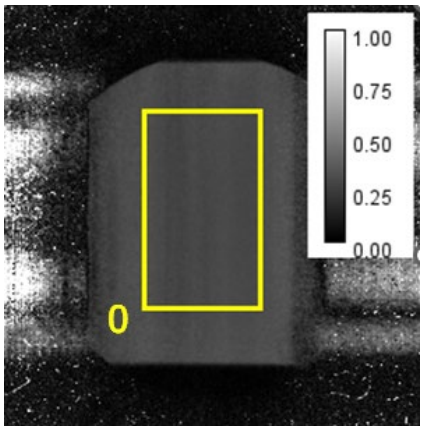

Fig.2 Measured visibility maps obtained with the newly fabricated G0 and G2.

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[2] Y. Seki, *et al.*, Rev Sci Instrum, 94, 103701 (2023)
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CO1-5

Structural Analysis of Viscosity Index Improver Molecules Using Small-Angle X-ray Scattering

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INTRODUCTION: Lubricating oils are necessary for friction reduction and high wear durability of sliding surfaces in machine components, and the development of the best oils is highly demanded by the industry. Viscosity index improver (VII) is a type of additive used to mitigate the decrease in viscosity of lubricating oil due to temperature rise. Classical textbooks explain that VII molecules work by changing their equivalent radius in the base oil according to the oil temperature. However, there are few papers investigating the equivalent radius of VII molecules by small-angle X-ray scattering (SAXS) and/or small-angle neutron scattering (SANS) [1], and there is still room for discussion about the behaviour and working mechanism of VII molecules in oil. This study aimed to investigate the radius of gyration of a VII polymer in base oil at different temperatures by SAXS, and the behaviour of the VII polymer was discussed.

EXPERIMENTS: To investigate the radius of gyration of VII polymer, a SAXS instrument (NANOPIX, Rigaku) with a Cu-Kα X-ray source (MicroMAX-007, Rigaku) and a semiconductor 2D detector (HyPix-6000, Rigaku) were used. The 1.2 mm-thick aluminum cell with optical windows made of 20 μm heat resistant engineering plastic film (Superio-UT, Mitsubishi Chemical) was used for the measurement. The cell temperature was successively increased to 25, 40, 60, 80 and 100ºC, and the final measurement was carried out again at 25ºC after cooling to check the degeneration of the VII molecule. Comb-shaped poly(methyl acrylate) (PMA) type VII was prepared as a model one used in the study. Squalane was used as a model base oil, and the concentrations of PMA in squalane were 0.5 mass%.

RESULTS: The SAXS profiles from squalane with comb-PMA type VII at each temperature are shown in Fig. 1. The profiles change as the temperature rises. Fig. 2 shows the Guinier plots, and the radii of gyration (R_g) and the former scattering intensities ($I(0)$). It was found that the comb-shaped VII molecule exhibited a gradual increase in R_g as the temperature rises. While this behaviour is similar to that of VIIs described in classical textbooks, it differs from that of another type of comb-shaped VII investigated previously. In the future study, we plan to separately discuss the role and behaviour of the main and side chains of comb-shaped VII molecules.

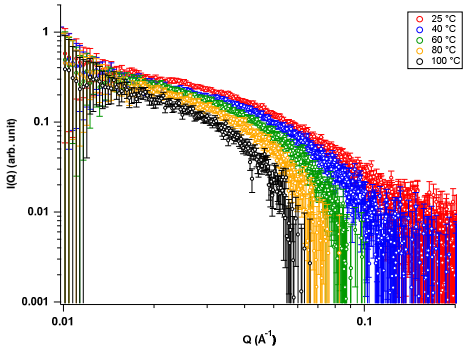

Fig. 1 SAXS profiles at each temperature.

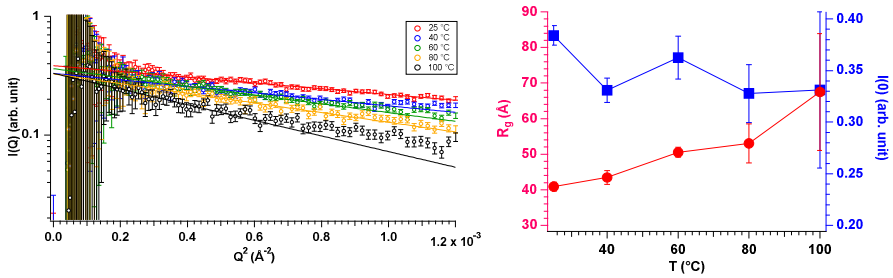

Fig. 2 Guinier plots (left) and the estimated R_g (right) at each temperature.

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CO1-6

Measurements with neutron interferometer and development of the half-mirror with suppressing distortion

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INTRODUCTION: Neutron interferometry is a powerful technique for studying fundamental physics. Numerous interesting experiments [1] have been performed since the first successful test of a single-crystal neutron interferometer [2]. However, the single-crystal interferometer is inherently not able to deal with a neutron that has a wavelength longer than twice its lattice constant. In order to investigate problems of fundamental physics, including tests of quantum measurement theories and searches for non-Newtonian effects of gravitation, the interferometry of cold neutrons is extremely important, since the sensitivity of interferometer for small interaction increases with the neutron wavelength. A large scale of interferometer also has the advantage to increase the sensitivity to small interactions. One of the solutions is an interferometer using neutron multilayer mirrors [3,4]. We can easily control parameters such as Bragg angle, reflectivity, and Bragg peak width by selecting the deposited material and tuning the bilayer thickness and the number of layers. We have demonstrated a multilayer interferometer for pulsed cold neutrons at the beamline 05 NOP in J-PARC MLF [5]. In the case of pulsed neutrons, the phase of interference fringes depends on neutron wavelength which is resolved by time-of-flight. Recently we tried to measure the neutron scattering lengths of ^{3}He, ^{4}He, and Xe gas with the interferometer. The gas cell which can be installed into the narrow gap of the interferometer was developed. These measurements provide important information for research in the physics of many-body systems of nuclei.

EXPERIMENTS AND RESULTS: In order to enlarge the number of neutrons, We are continuing to develop the neutron supermirrors which can reflect wide bandwidth of wavelength for the interferometer by using Ion Beam Sputtering facility in KURNS. In this study, we further reduced the number of layers compared to previous designs, resulting in a material with low reflectivity but extremely low deformation, that causes contrast loss of the interferogram. Figure 1 shows the reflectivity of the half mirrors with wide bandwidth, measured at MINE2 in JRR3. We will apply the mirrors to the interferometer.

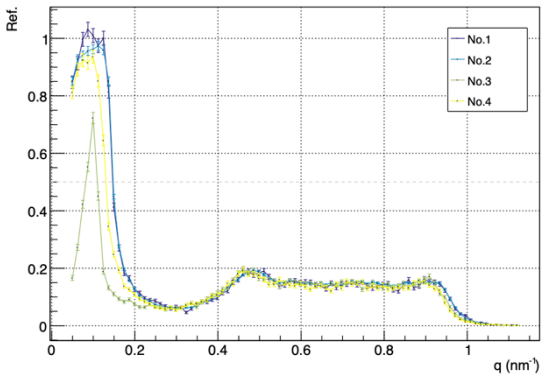

Fig. 1. Reflectivity of the half mirror with wide band of neutron wavelength measured at MINE2 in JRR3.

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