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Small-angle X-ray Scattering Beamline BL4C SAXS at Pohang Light Source II

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BL4C SAXS at the Pohang Light Source II is a small-angle X-ray scattering beamline based on an in-vacuum undulator insertion device, Si(111) DCM, and toroidal focusing mirror. The beamline normally provides high-flux synchrotron radiation X-ray sources with energies from 10.3 to 20.6 keV and a 100 μm (vertical) × 300 μm (horizontal) full width at half-maximum focal spot. The analysis of the SAXS data would be facilitated by means of useful ancillary equipment. The design of the beamline, the key components, and its role are described.

INTRODUCTION

Small-angle X-ray scattering (SAXS) is a fundamental and powerful tool for the structural analysis of polymers, metals, alloys, liquid crystals, and colloidal systems (Guinier et al., 1955; Glatter et al., 1982; Feigin et al., 1987; Roe, 2000). This technique provides information on the shape and size of macromolecules, characteristic distance of partially ordered materials, and size of pores (Yoon et al., 2008). The use of synchrotron radiation X-ray sources, with a high-flux photon and low divergence beam, enables a broader range of applications of the SAXS technique, such as in time-resolved measurements of bulk and powder samples, and in biological macromolecules in solution (Yoon et al., 2008). In scattering experiments, the specimen (e.g., particles of nanometer-scale size dispersed in solution or embedded in a bulk matrix) is exposed to X-rays and the scattered intensity I is recorded (Franke et al., 2009). For disordered systems, the random positions and orientations of particles lead to an isotropic intensity distribution $I(s)$, which depends on the magnitude of the scattering vector q ($q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle between the incident and scattered radiation, and λ is the wavelength of the X-ray beam source) (Franke et al., 2009). If the system includes identical non-interacting particles, for example, monodispersed dilute solutions of purified biological macromolecules, $I(s)$ is proportional to the scattering from a single particle averaged over all orientations (Franke et al., 2009). This permits one to obtain information on the overall shape and internal structure of particles at a resolution of 1–2 nm (Feigin et al., 1987; Svergun et al., 2003).

Recent progress in data analysis instrumentation and development has significantly improved the resolution and reliability of the models provided by using the SAXS technique (Svergun et al., 2003; Petoukhov et al., 2007). These advances have considerably increased the popularity of SAXS in the investigation of biological macromolecules in solution (Franke et al., 2009). The fundamental aim of structural studies in molecular biology is to establish a relationship between the structure, structural changes, and function of biological macromolecules. Over the past two decades, a large amount of structural information has been obtained through macromolecular crystallography and nuclear magnetic resonance (NMR) (Svergun, 1999; Gerstein et al., 2003; Sali et al., 2003). These high-resolution methods apply only in specific conditions: it is often difficult to grow high-molecular-weight (MW) crystal assemblies that are adequate for diffraction, and the application of NMR is fundamentally limited to low-MW proteins (Svergun, 1999). SAXS in solution can only produce low-resolution information (from approximately 1 to 100 nm) but is applicable in a broad range of conditions and particle sizes (Feigin et al., 1987). SAXS allows the analysis of biological macromolecules and their complexes in approximate physiological environments, and allows the direct examination of structural changes in response to changes in external conditions (Svergun, 1999).

In 2012, the Pohang Accelerator Laboratory (PAL) upgraded PLS to PLS-II and increased the electron energy in the storage ring from 2.5 to 3.0 GeV (Shin et al., 2013). PLS-II currently operates at 3.0 GeV with a storage ring current of 400 mA in top-up mode (Hwang et al., 2014). During the PLS-II upgrade, a 4C SAXS beamline was constructed for small- and wide-angle X-ray scattering (SAXS and WAXS) experiments to investigate the structure, structural changes, and relationship between the structure and function of a variety of samples, including nanostructured polymers, self-assembled organic/inorganic nanostructures, composite nanomaterials, biological macromolecules (proteins, DNA, and RNA) and their complexes in approximate physiological environments. The beamline has been made available to domestic and foreign users since 2012 (http://pal.postech.ac.kr/bl/4C/). In this paper, we summarize the current status of the main components and equipment of the 4C SAXS beamline.

BEAMLINE OVERVIEW

The 4C SAXS beamline is composed of a front-end (FE), optics hutch (OH), photon transfer line (PTL), and end-station (ES), each of which have important optical components. The main optical components of the 4C SAXS beamline are an in-vacuum undulator, Si(111) double crystal monochromator (DCM), and focusing mirror. The optical layout and specifications of the 4C SAXS beamline (BL) are illustrated in Figure 1 and Table 1, respectively.

For SAXS studies of soft materials such as biological macromolecules, high-flux X-rays are provided by means of a 1.4-m-hybrid, asymmetric-type in-vacuum undulator (IVU) with a magnet block of Sm2Co17, period length (λμ) of 20 mm, period number (N) of 70, and IVU gap range of 5–30 mm (SFA, Korea). For the operating conditions of the 3.0 GeV and 400 mA stored-beam-current PLS-II, the undulator provided a total X-ray power of up to 2.95 kW at K = 1.795.

The X-rays are monochromated ($\Delta E/E \approx 10^{-4}$) with a Si(111) DCM, which is located 18.0 m from the X-ray source (Vactron, Korea) (Figure 2). The function of the DCM is to select and transmit X-ray radiation of the desired photon energy from an incident white synchrotron radiation source. The DCM is designed to select energies between 10.3 and 20.6 keV. A HUBER goniometer (model 420) is mounted on the flange outside the vacuum for the rotation of the crystal and is used with a five-phase stepping motor to produce an angular resolution of 0.07″. Inside the flange, a rotational block is linked to the goniometer through a rotary feed-through with a ferromagnetic seal. The first flat crystal (70 mm wide × 50 mm long × 10 mm thick) is mounted on this block and its surface coincides with the rotational axis of the rotary feed-through. The second flat crystal (70 mm wide × 110 mm long × 10 mm thick) is mounted on a linear stage that is attached to the rotational block. The linear translation motion of the second crystal is used to produce a 25-mm fixed vertical beam offset with respect to the surface of the first crystal. In addition, the second crystal is attached to two picomotors with a resolution of 0.013–0.018″ and one piezo actuator with a resolution of < 0.00006″ for the fine tuning of pitch and roll. The first crystal surface is faced upwards and is placed below the surface of the second crystal. In addition, the first crystal with the liquid nitrogen (LN_2) channel is directly cooled, to dissipate the photon energy that is absorbed, by a constant supply of LN_2

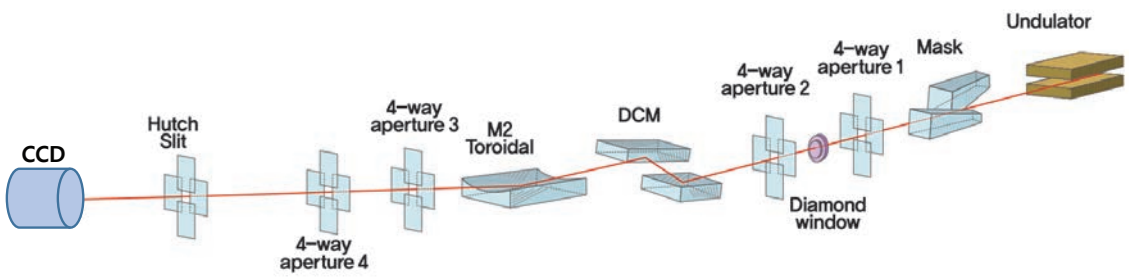

FIGURE 1 | Schematic layout of 4C small-angle X-ray scattering beamline (BL4C SAXS). Position of key optical components and equipment from the X-ray source are displayed.copyright.

TABLE 1 | Beamline specifications

Beamline name	BL4C SAXS
Source type	In-vacuum undulator (1.4 m short, 20 mm period)
Monochromator	Double crystal Si(111) liquid nitrogen (LN2)-cooled
Energy range	10.3–20.6 keV
Wavelength range	0.06–0.12 nm, currently 0.07 nm
Mirror	Vertical focusing toroidal, rhodium coated
Beam flux	1×10^{12} ph/sec
Beam size	100 (V) μm × 300 (H) μm
Resolution	200 nm–0.3 nm
Slits	Individually motorized blades of tungsten (W)
Sample-to-detector distance	5.0–0.2 m
Detector	Rayonix 2D SX 165
Experimental methods	Bulk, solution, liquid crystal, film, powder, sol-gel T-SAXS, T-WAXD

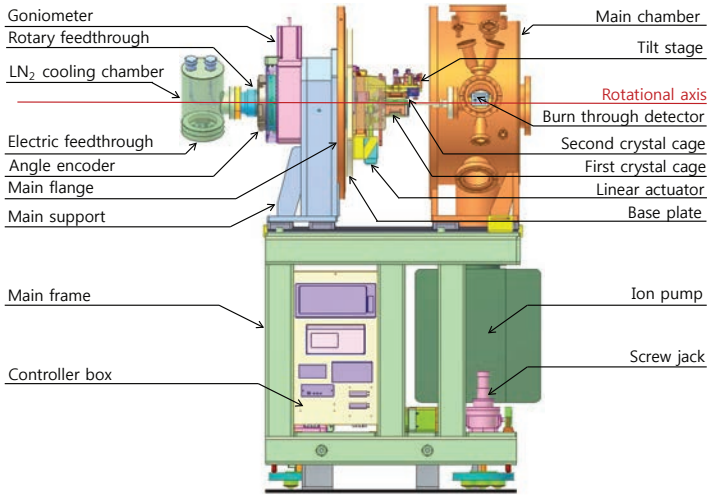

FIGURE 2 | Schematic of the Si(111) double crystal monochromator (DCM) installed in the optics hutch.

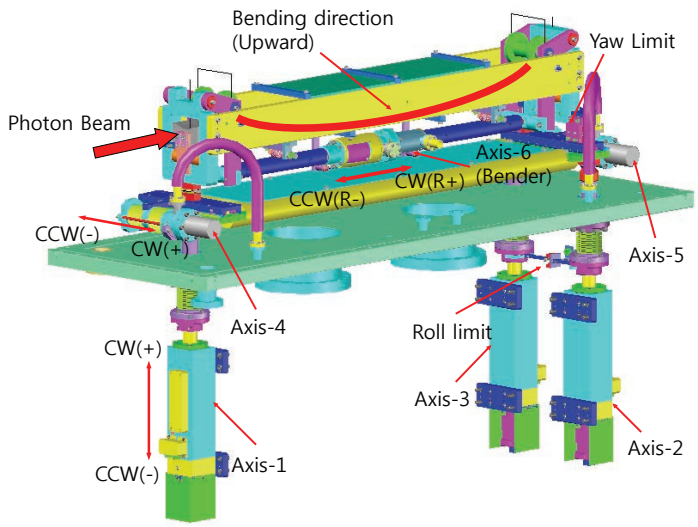

FIGURE 3 | Schematic and description of motor axis and conducting direction for the focusing mirror installed in the optics hutch.

from the LN_2 temperature control system. However, the second crystal is indirectly cooled by linking the block of the second crystal with that of the first one through the copper braid. Both DCM Si(111) crystals are mounted on a copper block by the lamination of an In-Ga eutectic and indium foil with a thickness of 125 μm to enhance the thermal conductivity. The crystal block is nickel-plated to prevent chemical reactions between the crystal block and InGa eutectic. In front of the DCM, a diamond window (Diamond Materials GmbH, Germany) with a diameter of 1 cm and thickness of 200 μm effectively reduces the thermal load on the first Si(111) crystal.

The monochromatic X-ray source obtained from the Si(111) DCM is focused with a vertical focusing toroidal mirror, which is located 21.3 m from the light source. The silicon mirror is supplied from Thales SESO SAS (France). The total mirror system was designed by PAL and manufactured by Vactron, as shown in Figure 3. The specifications of the focusing mirror for the BL4C SAXS are listed in Table 2. A focusing mirror is used at the BL4C SAXS to remove high-energy radiation and focus the X-rays at one spot. High energy X-rays over 23.0 keV are filtered from the spectrum by setting the incident beam at a grazing angle of less than 3.0 mrad. The focusing mirror is placed such that it reflects incoming X-ray beams from the mirror (1100 mm long × 75 mm wide × 55 mm high) upwards at an angle of 2.6 mrad. The beam is focused to a 100 μm (vertical) × 300 μm (horizontal) focal spot at the sample position (37.0 m from the X-ray source). The tangential radius of the mirror is approximately 5.9 km in the normal operating condition. The focusing mirror includes encoders for feedback on the accurate position of the mirror and photon beam position monitors for measuring the absolute position of the incoming X-ray beam with respect to the surface of the mirror. The surface of the mirror is coated with a 500-Å-thick rhodium coating layer, which is adequate to cover a wide energy range of 10.3–20.6 keV that the BL4C SAXS requires. A total of six motion actuators, including a five-phase harmonic (100:1) stepping motor, are used for optical alignment (i.e., linear vertical and horizontal translation) and fine tuning (i.e., pitch, roll, and yaw) regarding the incoming X-ray beam. As there is little thermal load because of the total reflection of the incoming X-ray beam, the focusing mirror is used without the cooling system.

The monochromatic X-ray beam reflected from the mirror passes through an evacuated PTL, Be window, ion chamber (FMB Oxford, UK), CCD linking shutter, attenuator, and vacuum chamber, and is focused onto a 2D detector, as shown in Figure 4. The SAXS detector is enclosed in an experimental hutch and all components are remotely controlled. A 3-axis straight-line motion sample stage is located right after the 4-way vacuum slit. Attenuators with various aluminum foil thicknesses can be located before the vacuum chamber to control the photon flux on the sample. An evacuated vacuum chamber with an adjustable sample-to-detector distance (5.0, 4.0, 3.0, 2.0, 1.0, 0.5, or 0.2 m) is mounted on a double optical rail between the sample and CCD stage. It is sealed at both ends with thin Kapton films and remains in a vacuum state (typically 5×10^{-3} Torr). The beamstop is made of tungsten and is located inside the vacuum chamber at the end, close to the Kapton window. Each piece of the

TABLE 2 | Specifications of a focusing mirror for the BL4C SAXS at PAL

Mirror surface shape	Horizontal toroidal
Mirror dimension	1100 mm long × 75 mm wide × 55 mm high
Effective area	1000 mm long × 55 mm wide
Vacuum chamber dimension	1530 mm long × 470 mm wide × 445 mm high
Mirror center height	1425 mm
Cooling method	No cooling
Vacuum degree	Less than 2×10^{-9} mbar
Reflection direction	Upward
Incidence angle	2.6 mrad
Sagittal radius	47.9 mm
Sagittal slop error	Less than 100 μrad
Tangential slope error	Less than 3 μrad
Tangential radius	5.9 km
Roughness	Less than 5 Å
Substrate material	Si single crystal
Coating material	Rhodium, 500 Å

experimental SAXS data is normalized to the transmitted X-ray beam intensity, based on the photocurrent transferred from the beamstop relative to the intensity of the transmitted X-ray beam. A 3-axis motor-controlled detector stage is installed right behind the vacuum chamber. As additional devices, copper screens and tungsten wire monitors for beam intensity and position monitoring are placed in the optics hutch and on the PTL.

Scattering data are collected by a Rayonix SX 165 CCD detector (Rayonix, USA). The SX 165 features a round, 165-mm diameter active area and a versatile, high-resolution CCD chip. Incident X-rays are converted by a Gd_2O_2S phosphor screen into visible scintillation photons that are guided to the CCD image sensor with a fiber-optic taper demagnification ratio of 2.7:1. The CCD that is composed of four read-out channels provides extremely low readout noise at high readout speeds with a dynamic range of 16 bits. For on-chip binning of 2×2, the number of pixels in the image and pixel size are 2048×2048 and 80 μm, respectively, resulting in a read-out time of 2.5 s and read-out noise of 13 e^-/pixel. The CCD chip in the SX 165 is cooled to –80°C through closed-circle refrigeration and is protected inside a sealed vacuum chamber so that the resulting dark current is less than 0.01 e^-/pixel/s. To prevent image smearing during the readout, an electromagnetic fast CCD shutter that is triggered by the CCD controller was installed between the ion chamber and attenuator, as shown in Figure 4.

SAMPLE STAGE

The samples are mounted manually, and the temperature over 25–400°C is controlled by a cartridge-based 2404 temperature controller (Eurotherm, USA), and over 0–200°C by an FP50-HL refrigerated circulator (JULABO, Germany). The beamline has two types of sample cells, a Kapton film-based flat cell with a 1.0-mm thickness for bulk and powder samples, and a quartz capillary cell with a 1.5-mm diameter and 0.01-mm wall thickness for solution samples (Figure 5). Recently, we fabricated multi-cooling and heating sample stages for solid and solution samples to improve the efficiency and effectiveness of experimentation.

BEAMLINE SOFTWARE

Data collection from a Rayonix SX 165 CCD detector is controlled by BL4C SAXS data collection software developed by PAL based on network-attached storage (NAS) and the experimental physics and industrial control system (EPICS) (http://www.aps.anl.gov/epics/), programmed on Linux. The software for all the optical components of the BL4C SAXS has an interactive GUI and is designed to run on Linux and partly on Windows, with the assistance of the EPICS. Data processing such as 2D image background correction, 1D radial averaged data, scaling, and 1D azimuthal data acquisition is carried out by data processing software that PAL developed based on the Python programming language running on Windows.

BEAMLINE EQUIPMENT

We have a user laboratory for sample preparation, purification, and characterization, next to the beamline. It includes high-performance liquid chromatography (Shimadzu, Japan) and Eclipse DUALTEC field-flow-fractionation instruments (Wyatt, USA) for separating proteins, biopolymers, polymers, and nanoparticles. A Zetasizer Nano-ZS90 dynamic light scattering (Malvern, UK) system, for measuring particle size at 90°

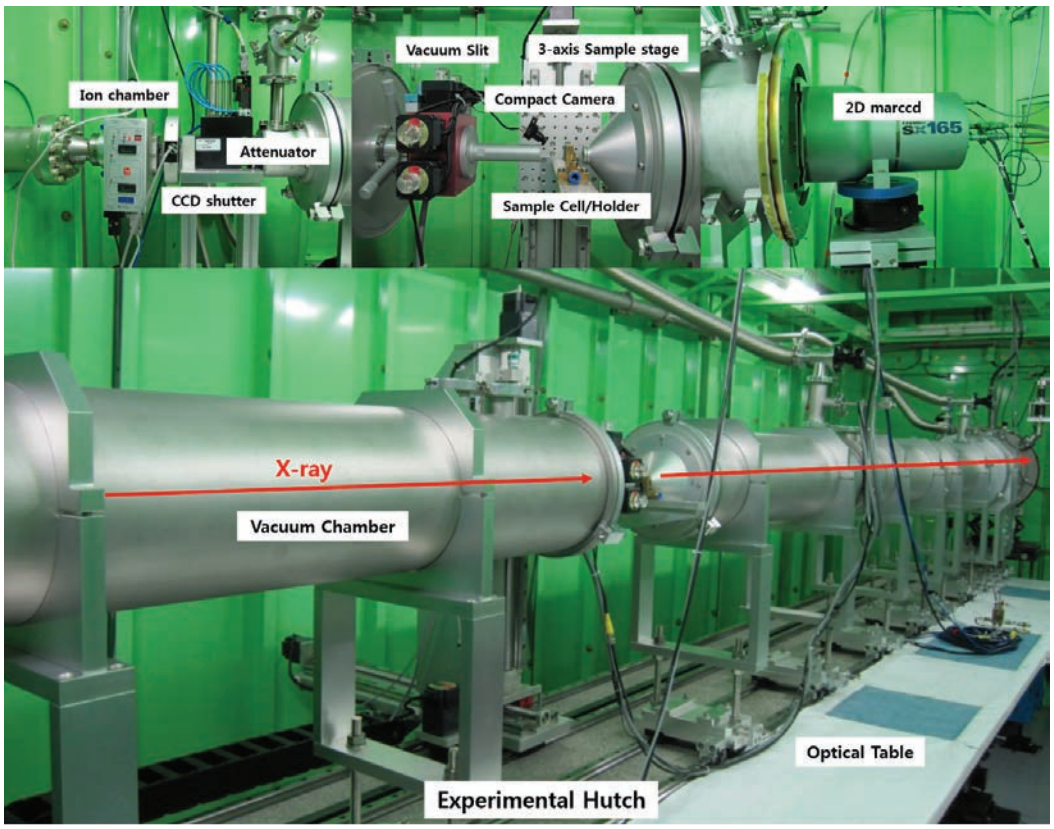

FIGURE 4 | Photograph of experimental devices in the experimental hutch of the end-station.

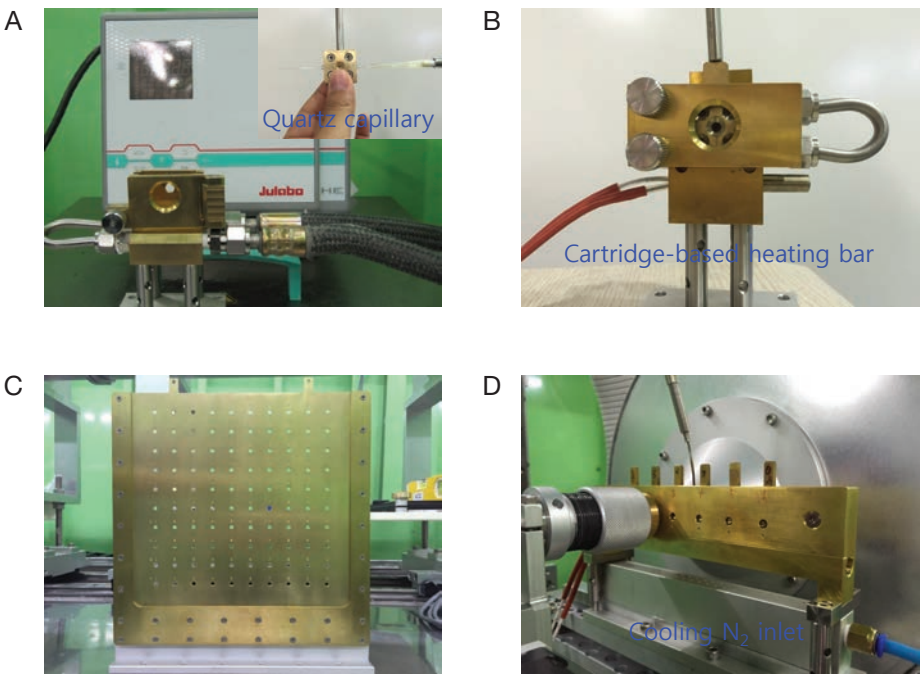

FIGURE 5 | Photograph of experimental sample stages in the experimental hutch of the end-station. (A) Cooling and heating sample stage for solution. (B) Cooling and heating sample stage for sol-gel. (C) Multi (100 holes) sample stage for bulk and powder. (D) Multi (6 holes) heating sample stage for bulk and powder. al., 2015) copyright.

scattering angles, and a DAWN Heleos II multi-angle light scattering instrument (Wyatt, USA), for characterizing absolute masses and sizes of molecules, are installed in the room. The NanoDrop 2000C UV-Vis spectrophotometer (Thermo scientific, USA) can measure a variety of sample types over a wide spectral range of 190–840 nm. The analysis of the SAXS data would be greatly facilitated if the sample was characterized by complementary techniques that the beamline serves. The beamline is also equipped with a refrigerated centrifuge, water purification system, ultra-low temperature freezer, cold storage, ultrasonic cleaner, electronic balance, pipette, dry bath, bench mixer, and hot plate.

CONCLUSIONS

The 4C SAXS beamline at PLS-II was designed and constructed for small-angle X-ray scattering experiments in 2012 and has been operated by domestic and foreign researchers in various experimental setups. The BL-4C SAXS uses tunable high-brilliance synchrotron radiation sources to obtain remarkable research results as the combination of the in-vacuum undulator insertion device, Si(111) DCM, and focusing mirror provides highly stable, high-flux synchrotron radiation. In addition, the ideal X-ray CCD detector with excellent reliability collects a huge amount of data when compared to laboratory-based X-ray sources. The BL-4C SAXS software has a user-friendly, Window-based GUI, and several ancillary equipment in the laboratory allow users to perform biophysical studies of various samples. In the near future, we are planning to introduce a time-resolved SAXS technique combined with size exclusion chromatography, enabling us to perform SAXS measurements while purifying polydispersed samples like oligomeric, mixture, incomplete biomolecules, and nanoparticles by size and molecular weight.

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AUTHOR INFORMATION

The authors declare no potential conflicts of interest.

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