NE-CAT Communications

A Biannual Newsletter of the Northeastern Collaborative Access Team Winter 2021

Message from the Director

Steve Ealick

It has been a long time since the last newsletter from NE-CAT. There have been many changes. We have transitioned from a P41 grant to a P30 grant. We re-designed our website. We held a memorial symposium in New York City to honor Associate Director, K. "Raj" Rajashankar whom we lost in 2019. We have added Dr. Ali Kaya as a new member of our staff. We have continued to push science forward while working remotely during the COVID-19 pandemic. By the time this newsletter is released, it is possible that a sense of normalcy will have returned. At this moment, the APS remains in limited operations and all users are required to collect data remotely. Yet, NE-CAT continues to operate at almost full capacity on both beamlines through our custom web-based remote data collection GUI. Visit our website to schedule beamtime, find links to RAPD and REMOTE, for the most recent updates and events or contact our staff: https://necat.chem.cornell.edu/

Beamline Developments

1. EIGER2 X in Use

In the last newsletter, we announced the arrival of the EIGER detector. After the arrival of the EIGER, NE-CAT upgraded our network and computing infrastructure to handle the anticipated additional data transport and computational load. Included in the upgrade are: InfiniBand switches and host bus cards, FDR InfiniBand active optical cables, new fiber optic switches, an EIGER processing unit, additional compute cluster nodes, and a faster core Ethernet networking switch. These items create an even faster high-speed network and increased data processing power. Old obsolete network infrastructure on both beamlines was also upgraded concurrently and the entire network was put to the test when the EIGER detector was commissioned on 24-ID-E over three days during the 2017-2 run.

In the fall of 2019, we received the EIGER2 X 16M from Dectris. In 2020, just before the nation locked down to prevent the spread of COVID-19, NE-CAT commissioned the EIGER2 X on 24-ID-C. It replaces the PILATUS, which while still functional, is a slower pixel array detector with fewer pixels (as seen in the comparison chart of the PILATUS vs the EIGER in the Summer 2016 newsletter). The EIGER2 X 16M has 16 million pixels, a frame rate of 130 Hz, and an active area

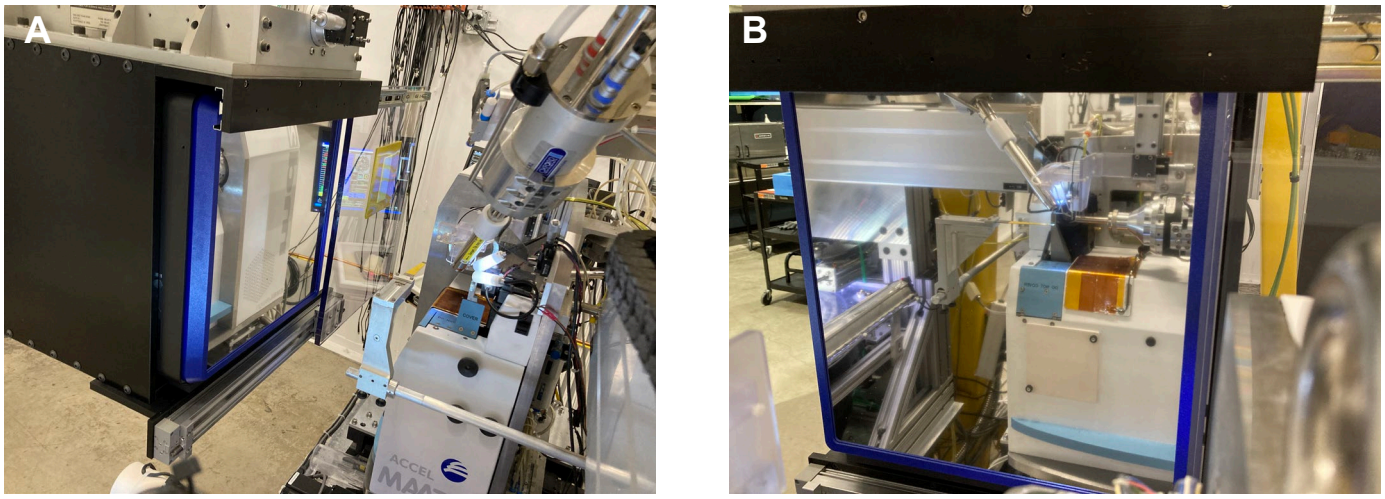


Fig. 1. EIGER2X installed and in use on 24-ID-C. A. Side View. B. Front view of EIGER2 detector face showing reflection of MD2 microdiffractometer and sample area.

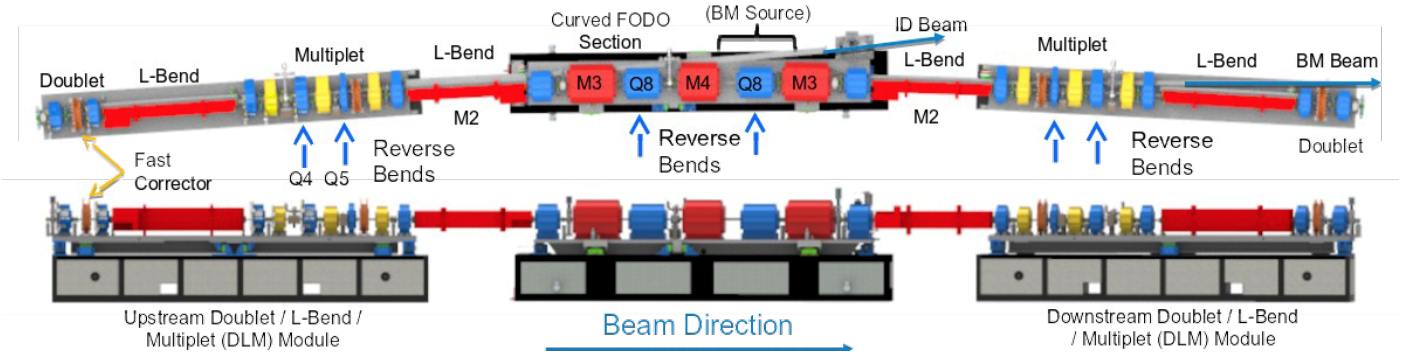


Fig. 3. One cell of the new multi-bend achromat at ESRF. A similar design has been proposed for the APS-U. (Xiao, A (2018). The Upgrade of the Advanced Photon Source. 9th International Particle Accelerator Conference.)

of 311 x 328 mm^2. While the EIGER2 modules are slightly smaller than the EIGER modules, the EIGER and EIGER2 have almost the same number of pixels, active area, and frame rate; this allows for quick and easy integration of the new detector into our beamlines and beamline software.

Most importantly, the EIGER2 has an 100Gb InfiniBand output to our upgraded network. This is only possible because Dr. Jon Schuermann worked closely with Dectris during the construction of our EIGER2. Due to his diligence, Dectris now provides the 100Gb InfiniBand connection as an option to other purchasers. In addition, the EIGER2 has the option of being upgraded to collect at 400 Hz which is not possible with the EIGER. The EIGER2 will be a detector to keep 24-ID-C at the cutting edge now and for years to come.

Once commissioned in run 2020-1, the EIGER2 was immediately available to users. Due to COVID restrictions, all remote users, without realizing, have been using the EIGER2 to collect data for the last year and the rollout has been seamless.

2. APS-U

For several years, the APS has been planning for a massive upgrade to the entire complex, known as APS-U. The entire accelerator complex will be replaced with a new system of greatly reduced horizontal emittance yielding dramatic increases in the brilliance, longitudinal and temporal coherence of undulator X-ray beams; less than 50 pm is expected. This will be accomplished by replacing the current dipole-based storage ring with a multi-bend achromat-based accelerator, similar to those employed in the MAX IV and upgraded ESRF synchrotrons. In addition, the existing klystron radiofrequency (RF) system used to accelerate and inject electrons into the storage ring will also be replaced with a solid-state amplifier-based RF system. Beamline front ends, including the one for NE-CAT, will be upgraded to handle the greater thermal loading imposed by the APS-U. The new accelerator will have a critical energy of 6 GeV (down from 7 GeV), will eventually operate at 250 mA (up from 100 mA), have a symmetric beam cross section (compared to the current 50:1 horizontal to vertical profile), and yield a 10 to 100-fold increase in X-ray brilliance to undulator beamlines. All APS undulators will also be upgraded for best fit with each sector's experimental requirements and the optical properties of the new accelerator.

The APS-U project is currently at the DOE CD3 level of execution. As announced at the 2021 User's Meeting in May, the APS will shutdown in April 2023 to begin the year-long process of deconstruction of the old accelerator and construction of the new multi-bend achromat. Recommissioning of the NE-CAT beamlines will start once the new accelerator provides positionally stable beam at 25 mA (expected fall 2024). User operations will be restarted when stable 50 mA operation of APS-U is achieved, and the NE-CAT beamlines have completed recommissioning. We currently expect to re-commission 24-ID-E first, followed by 24-ID-C.

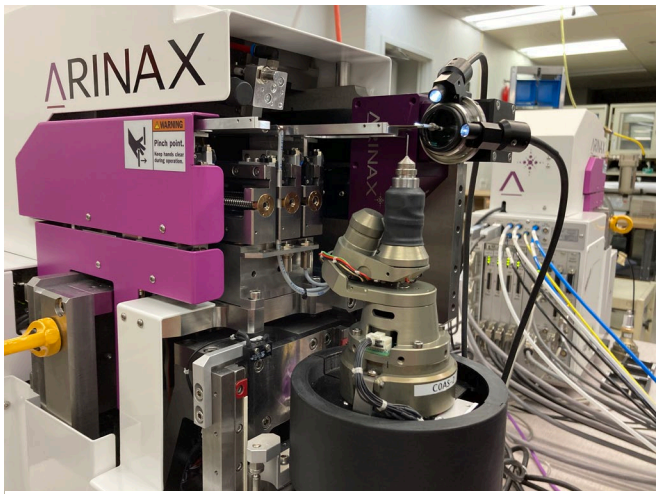


Fig. 2. MD3 diffractometer on the dry lab bench.

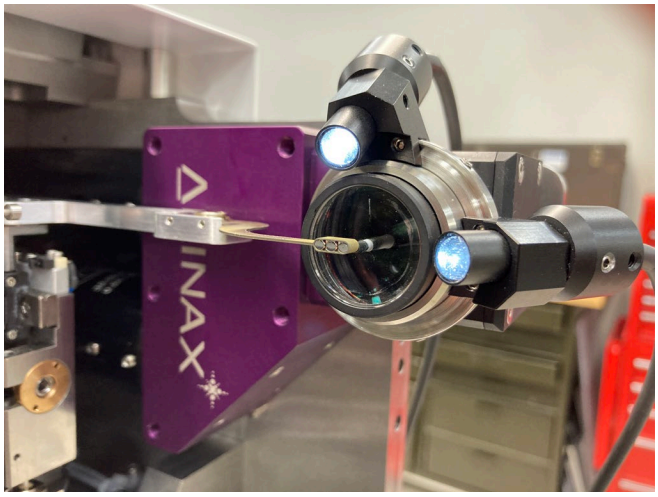


Fig. 4. Closeup of the triple aperture which will assist in shaping the microbeam once the MD3 is installed on a beamline.

3. MD3 Microdiffractometers

Currently on both beamlines, NE-CAT has installed MD2 microdiffractometers. These machines provide high magnification visualization of microcrystals, and high precision sample alignment, and a low sphere of confusion during sample rotation. However, these instruments have have been in use over 12 years at NE-CAT and are showing signs of their age. In anticipation of the APS-U, NE-CAT has ordered two MD3 microdiffractometers from Arinax. The MD3 microdiffractometer has a smaller sphere of confusion, allowing for greater stability of micro-crystals and greater accuracy in sample alignment.

The MD3 microdiffractometer also has a vertically-oriented spindle instead of the horizontally-oriented spindle on the existing MD2. This will necessitate redesign of the sample automounters, reconfiguration of the beamlines, new software to handle sample delivery, and new software to handle data collection. The current plan is to replace the aging MD2 microdiffractometers with new MD3 microdiffractometers. 24-ID-C will receive its MD3 during the APS-U while 24-ID-E re-commissions with the existing beamline structure intact in order to be immediately available to users and have its MD3 installed in the year following the APS-U.

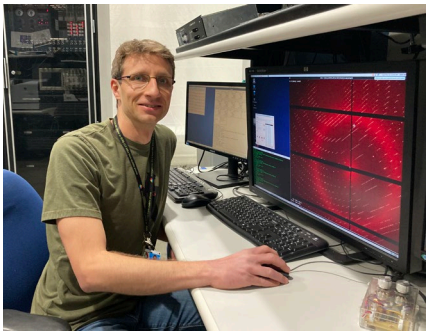
Both MD3 microdiffractometers arrived from Arinax at the end of November 2021. They are currently undergoing system and calibration checks in the dry lab by technicians from Arinax to determine if the machines arrived safely and without damage from Europe.

4. New Safety Officer

After many years of service, Dr. N. Sukumar has stepped down as NE-CAT Safety Officer. Dr. David Neau has taken over the mantle of Safety Officer. Following guidelines and directives from the APS and Argonne National Laboratory, the Safety Officer oversees the safety protocols at NE-CAT. The Safety Officer provides guidance to NE-CAT staff on safety procedures, provides personal protective equipment, and ensures that training requirements are met. David will be assisting all NE-CAT users in filling out Experimental Safety Assessment Forms and in being up to date with all ANL and APS training requirements. He is also the default staff scientist in charge of hazardous material shipping. We encourage all users to address dewar shipments to David or your support scientist (https://necat.chem.cornell.edu/shipping).

David Neau received his Bachelor of Science from Duke University in 1998 and his Ph.D. in Biological Sciences from Purdue University in 2004 where he specialized in protein crystallography and structural biology. He was the assistant beamline manager for the Gulf Coast Protein Crystallography Consortium at the Center for Advanced Microstructures and Devices at Louisiana State University prior to joining NE-CAT in 2008. In addition to his new Safety Officer duties, David provides user support, and is part of the RAPD development team.

5. NE-CAT New Hire



Just as the APS entered COVID lockdown in March 2020, NE-CAT added a new staff member at the level of postdoctoral associate, Dr. Ali Kaya. Ali Kaya obtained his PhD in Biotechnology from Ankara University Biotechnology Institute, Ankara, Turkey, in 2009 working on β2-adrenergic receptor ligands. He then moved to the Department of Pharmacology at Vanderbilt University where he investigated Rhodopsin-G Protein interactions with phospholipids in bicelles. Before moving to NE-CAT, he worked with Dr. Heidi Hamm and Dr. Tina M. Iverson at Vanderbilt University on understanding the dynamics and activation mechanism of heterotrimeric G

proteins by using different biochemical, biophysical, and crystallographic research techniques.

6. Posters Wanted

In the past 10 years, NE-CAT has transitioned from mainly on-site users to mainly remote users. Though we have fewer visitors, the APS remains a hub of research and we would like to see the research of our graduate student and postdoctoral researchers on our walls. If you have a recent poster (or poster file), we have wall space to show it off. Let your support scientist know that you have a poster that you wish to share with us, and it might not only show up on our walls but in a future newsletter!

Research Highlights

Crystal structures of OLD family nucleases reveal evolutionary insights into anti-phage defense machineries

Joshua Chappie, Assistant Professor, Department of Molecular Medicine, Cornell University, Ithaca, NY.

Antibiotic-resistant bacteria represent a rising threat to human health. A lack of new drugs targeting these dangerous 'superbugs' has revived the clinical use of lytic bacteriophage viruses (phages) as therapeutic agents. Successful application of phages to treat multidrug-resistant infections in human patients has highlighted the feasibility of this strategy in recent years.[1,2] Despite these efforts, bacteria have evolved a diverse array of defense systems to protect themselves against viral infection and phage-mediated killing. Restriction modification systems, modification-dependent restriction systems, CRISPR, abortive infection systems, CBASS immunity, and retrons collectively represent an endogenous impediment to phage therapy. Understanding the structures and fundamental molecular mechanisms of these defense machineries is therefore an important step for improving the effectiveness and general applicability of phage-based approaches.

The explosion of genomic data and advancement of bioinformatic analyses have identified many new players in the ongoing arms race between bacteria and phages. Among these are the OLD family nucleases. OLD enzymes were first described in phage genetic experiments (the founding P2 *old* gene being named for the 'overcoming lysogenization defect' plaquing phenotype)[3] but homologs have since been shown to be widely distributed across in bacteria, archaea, and plasmids. Emerging evidence suggests that *old* genes provide broad protection again a diverse spectrum of phages, with some conferring this activity directly and others functioning indirectly as effectors for retron systems.[4–6] OLD homologs can be subdivided into two classes based on their genomic context: Class 1 enzymes exist as single genes while Class 2 enzymes appear in tandem with a UvrD/PcrA-like repair helicase. Both classes share a common domain arrangement consisting of an N-terminal ABC ATPase, a dimerization domain, and a C-terminal Toprim domain (Fig. 5A). Previous *in vitro* characterization of P2 OLD demonstrated measurable nuclease and ATPase activities consistent with this organization.[7] Prior to our work, however, no concrete structural information existed and the catalytic machinery had yet to be defined.

To gain insights into OLD structure and function, a stellar team of graduate students and undergrads (Fig. 2) set about purifying and crystallizing different constructs of both Class 1 and Class 2 OLD homologs. These valiant efforts were spearheaded by Carl Schiltz and yielded structures of the catalytic C-terminal regions (CTR) of Class 2 OLD proteins from

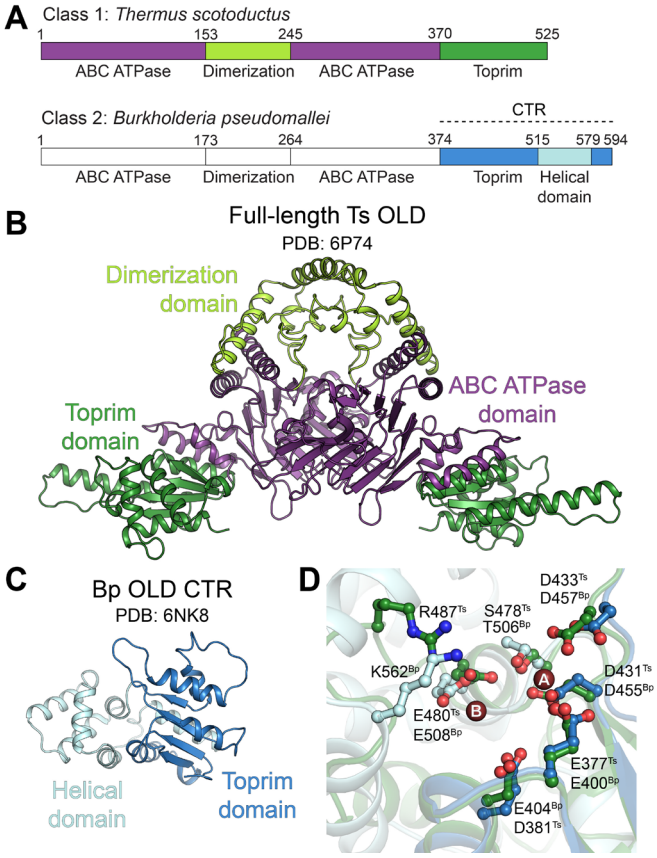


Fig. 5. Structures of OLD family nucleases. A. Domain arrangement of representative Class 1 and Class 2 OLD homologs. B. Structure of full-length Ts OLD (Class 1). C. Structure of Bp OLD CTR (Class 2). D. Conserved active site organization of OLD nucleases. Ruby spheres labeled 'A' and 'B' denote positions of the bound magnesium ions present in Bp OLD CTR structure. Invariant side chains providing metal coordination are spatially conserved among all OLD homologs.

Burkholderia pseudomallei (Bp) (PDB: 6NK8) and *Xanthomonas campestris* pv. *campestris* (Xcc) (PDB: 6NJW) at 2.24-Å and 1.86-Å resolution, respectively, and the full-length structure of a Class 1 OLD protein from *Thermus scotoductus* (Ts) (PDB: 6P74) at 2.20-Å resolution (Fig. 2B and 2C). The NE-CAT 24-ID-C tunable beamline was instrumental in determining these structures, allowing us to obtain SAD data and experimental phases from a number of different derivatives (e.g. Hg, Pt, Ir, Pr, I, and Sm).

The Bp and Xcc CTR structures provided the first glimpses into how OLD enzymes cleave DNA. Both showed a bipartite architecture comprised of a Toprim domain with altered topology and a unique helical domain that resembles bacterial controller proteins. Two magnesium ions were present in the active site of Bp OLD, coordinated by invariant acidic side chains from both domains in a geometry consistent with a two-metal catalysis mechanism for DNA cleavage (Figure 3D). In this configuration, metal A activates the nucleophile for attack on the scissile phosphate while metal B stabilizes the transition state intermediate. Positioned nearby was an additional invariant lysine that was poised to deprotonate the leaving group and/or provide additional charge compensation. Mutagenesis revealed that either metal alone permits nicking of DNA substrates while full nuclease activity requires proper coordination of both metals and the presence of the conserved lysine. Frustratingly, sequence alignments failed to pinpoint the corresponding residues in Class 1 enzymes. This discrepancy was ultimately resolved when we were able to compare the CTR structures with the Ts OLD coordinates. Structural superposition indicated that Class 1 enzymes lack the helical domain but contain a spatially conserved set of residues that maintain the layout of the active site (Fig. 5D). Mutagenesis confirmed the importance of these amino acids thereby establishing a unified mechanism for nuclease activity among different classes of OLD enzymes.

The Ts OLD structure also proved valuable for understanding OLD ATP hydrolysis. Using the DALI structural comparison server, we found that genome maintenance proteins like Rad50 and SMC complexes constitute the nearest structural homologs of OLD ABC ATPase domains. Further scrutiny indicated that OLD enzymes contain a number of identifiable alterations to the canonical ABC catalytic motifs. This includes a degenerate signature sequence and histidine substitutions in the Q loop and D loop. These variations are conserved across classes and essential for activity both *in vitro* and *in vivo*, making them defining features that distinguish OLD nucleases among the ABC superfamily.

Manuscripts describing our findings were published in *Nucleic Acids Research*.[8,9] Our characterization of additional OLD homologs and examination of different conformational and substrate-bound states continues, with NE-CAT serving as an integral resource for our crystallographic studies.

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Staff Activities

Fig. 6. Chappie lab contributors to the OLD nuclease project (past and present). Top row: Joshua Chappie, Carl Schiltz, Myfanwy Adams. Bottom row: April Lee, Christopher Hosford, Edward Partlow.

Talks

Perry, K. “Featured Friend,” Molecular Biology and Chemistry Seminar Series, Christopher Newport University, Virtual, October 13, 2021.

Banerjee, S. “Structural Biology in Drug Discovery,” Calcutta University, Kolkatta, India, Sept. 11, 2021.

Banerjee, S. “The Mathematical Lens - Fourier Transform,” University of Burdwan, Bardhaman, India, May 28, 2021.

Murphy, F. “RAPD data analysis at NE-CAT,” Current and Future Trends in Macromolecular Crystallography Experiments: Focus on Automation, High Data Rate Analysis and User Interfaces, MCE 2021, Brookhaven National Laboratory, Virtual, March 16-18, 2021.

Capel, M. “New endstations at NE-CAT: MD3UP microdiffractometer and 30 puck ALS-style loader,” Current and Future Trends in Macromolecular Crystallography Experiments: Focus on Automation, High Data Rate Analysis and User Interfaces, MCE 2021, Brookhaven National Laboratory, Virtual, March 16-18, 2021.

Posters

N. Sukumar, I. Kourinov, M. Capel, J. Withrow, S. Sukumar and V.L. Davidson, “Ultra-high resolution and charge-density studies on type-I copper protein, amicyanin, from *Paracoccus denitrificans*”, Biophysical Society Annual Meeting, Virtual, February 22-26, 2021.

Publications

Shi, F., Mendrola, J. M., Sheetz, J. B., Wu, N., Sommer, A., Speer, K. F., Noordermeer, J. N., Kan, Z. Y., **Perry, K.**, Englander, S. W., Stayrook, S. E., Fradkin, L. G., and Lemmon, M. A. (2021) ROR and RYK extracellular region structures suggest that receptor tyrosine kinases have distinct WNT-recognition modes, *Cell Rep 37*, 109834. PMID: 34686333.

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Moeller, N.H., Shi, K., Demir, Ö., **Banerjee, S.,** Yin, L., Belica, C., Durfee, C., Amaro, R.E., and Aihara, H. (2021) Structure and dynamics of SARS-CoV-2 proofreading exoribonuclease ExoN, *bioRxiv*. PMID:33821277. **PMC8020977**

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