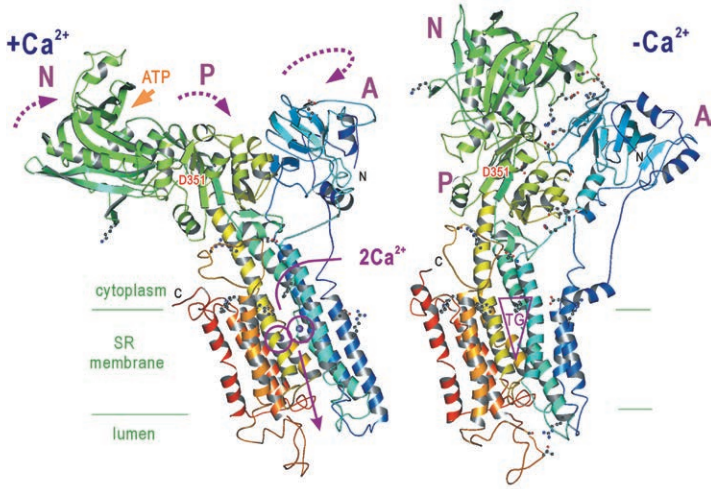
Life Science — Protein Crystallography

Insight into the mechanism of active transport by calcium pump

SPring-8 played a vital role in the recent structure determinations of the sarcoplasmic reticulum (SR) calcium pump in the calcium bound and unbound states. The ATP-driven calcium pump is an integral membrane protein (molecular weight of 110 k) that relaxes muscle cells by pumping calcium released during contraction back into the sarcoplasmic reticulum. The crystals were thin (<20 µm; Ca^{2+}-bound form) or had a very large unit cell dimension (nearly 600 Å; Ca^{2+}-unbound form). Hence, the use of very bright and highly parallel X-ray beam available in undulator beamlines, such as BL41XU (Structural biology I) and BL44XU (Protein Institute, Osaka University), were essential to these structure determinations.

These studies have revealed that the binding of calcium alone accompanies a surprisingly large-scale rearrangement of both transmembrane and cytoplasmic domains. and that the ion pumps work like mechanical pumps at an atomic scale. Also, the structure of a very strong inhibitor, thapsigargin (TG), bound to this pump was determined and may serve as a template for drugs targeted for membrane proteins. Calcium is a fundamental and ubiquitous factor in the regulation of intracellular processes. Therefore, the atomic structures of the calcium pump in different states have a tremendous impact on many fields, including medical treatment for myocardial diseases and cancer.



BL41XU Chikashi Toyoshima (University of Tokyo)

Life Science — Protein Solution Scattering

Structural change of calmodulin molecule caused by calcium binding

Calmodulin is a small protein with a molecular weight of 17,000 that is expressed in almost all eukaryotic cells and plays a role of transporting intracellular information. When the calcium concentration in the cell is increased by an external stimulation, calmodulin binds calcium ions and then binds to other proteins such as enzymes, and causes various changes in the cell. Upon binding calcium, the structure of the calmodulin molecule changes, allowing it to bind to other proteins. X-ray crystallography revealed that the calmodulin molecule is extended in the absence of calcium, whereas it becomes globular upon binding calcium ions. However, this change occurs in a short time (milliseconds) and its details remained unclear. In this study, the structural change of the calmodulin molecule was clarified by the small-angle scattering technique using intense X-rays from the undulator beamline BL40XU.

When calmodulin molecules were dispersed in solution and the calcium concentration was rapidly increased using a chelating agent that released calcium upon laser irradiation, the radius of gyration (an indicator of molecular size) of the calmodulin molecules decreased by ～25% in ～10 ms (Fig. 1). This indicates that the calmodulin molecules bound calcium ions and became compact. When a peptide was present as a binding partner in the solution, the compact form was stabilized and maintained (Fig. 1, white). Without the binding partner, however, the calmodulin molecules returned to the original extended state in ～150 ms (Fig. 1, black).

The existence of the compact structure observed immediately after calcium binding (Fig. 2) was demonstrated for the first time. This structure corresponds to the crucial state in the transmission of the calcium signal by calmodulin molecules through binding to other proteins. This finding will lead to clarification of cellular functions and to development of agents that target these proteins.

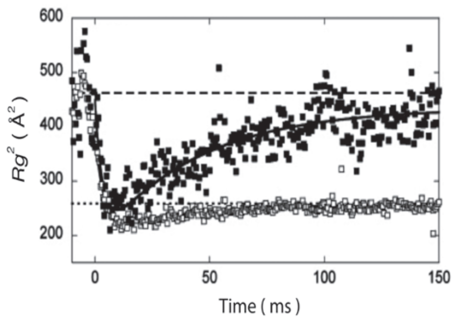


Fig. 1 Change in radius of gyration (Rg) of calmodulin molecules when calcium concentration was increased at time 0

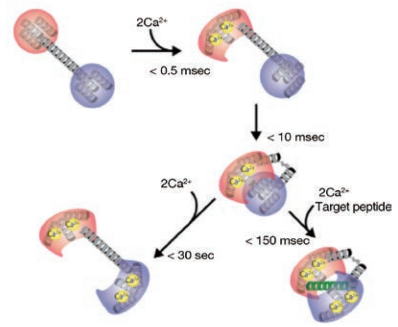


Fig. 2 Structural change of a calmodulin molecule upon calcium binding

BL40XU Yoshiteru Yamada, Hiroyuki Iwamoto, Naoto Yagi (JASRI)

Material science — CT+XRD

Direct Observation of External Force-Induced Changes in Internal Structure of Steel Sheet for Next-Generation Vehicles —Development of Combined X-ray Computed Tomography Analysis Technique—

Transformation-induced plasticity (TRIP) steel is coming into use as the steel sheet for next-generation vehicles. It has a unique feature called "phase transformation", namely, the change in metal structure caused by an external force. TRIP steel consists of ferrite and 20–30% of a metastable phase called retained austenite dispersed across the ferrite phase. The soft retained austenite phase is transformed to a hard martensite phase when an external force is applied. However, the phase transformation in TRIP steel must be observed and analyzed nondestructively because the phase transformation is easily induced even by grinding and machining. We developed a multimodal assessment technique by combining (1) X-ray nanotomography for the nondestructive, direct visualization of the phase transformation behavior in TRIP steel and (2) pencil-beam diffraction tomography for the measurement of crystallographic orientation and dislocation density. The spatial resolution of X-ray nanotomography is 0.16 µm, and the diameter of the X-ray beam used in pencil-beam diffraction tomography is 3 µm, which enables the observation and analysis of a very fine material structure. Figure 1 shows the schematics of the experimental setup. Switching between these methods takes 2–3 min and can be performed by just pressing a button. In this study, this technique was applied for the first time to the in situ observation of steel under external loading. As a result, the phase transformation, deformation, and rotation behaviors of individual austenite grains were clearly observed in three dimensions. Figure 2 shows the transformation behavior of a particular crystal grain for which the crystallographic orientation was determined. Previously, it was impossible to obtain design guidelines for the optimal microstructure because only average information from a wide area of steel could be achieved. However, the interactions between the individual retained austenite grains were directly visualized in this study, which provided clear guidelines for microstructure design.

The direct monitoring of phase transformation phenomena by the multimodal assessment technique will provide an accurate understanding of shock absorption and fracture properties, leading to a microstructure design for optimum shock absorption and fracture properties.

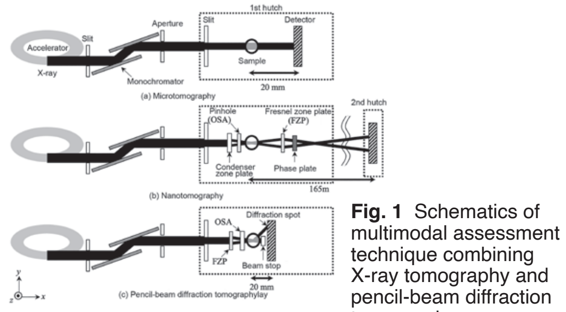


Fig. 1 Schematics of multimodal assessment technique combining X-ray tomography and pencil-beam diffraction tomography.

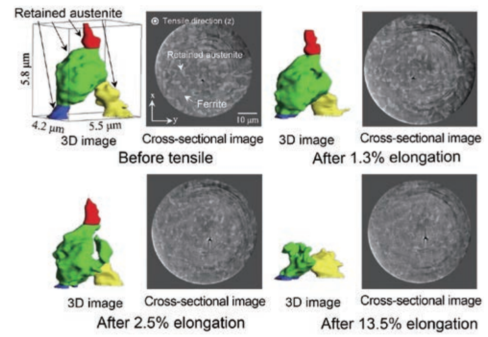


Fig. 2 Phase transformation behavior of retained austenite grains. The figure also shows the transformation behavior in the cross-sectional images of the grain during the same loading steps. The transformation of retained austenite grains occurs locally but not uniformly.

BL20XU Kyosuke Hirayama (Kyoto University), Hiroyuki Toda (Kyushu University)

Material science — Femtosecond Time-Resolved X-ray Diffraction at SACLA

Observing Instantaneous Dislocation in Steel During Ultrafast Heating

The heating and cooling rates during thermal treatment in the manufacturing of steel are extremely important parameters that determine the properties of these materials. However, the physical and kinetic understanding of atomic diffusion and structural changes with rapid changes in temperature has not yet been clarified because of difficulties in their observation. In particular, for the development of manufacturing techniques for high-quality materials, it is crucial to understand and precisely control dislocations (crystal defects) during microstructural changes from martensite to austenite (phase transformation). The ultrafast structural changes caused by an extreme increase in temperature are unknown. Important insights can be gained by observing these changes that will lead to the production of high-performance and high-quality steel. Although dislocations have been observed at a heating rate of 2–3 °C/s, it has been difficult to observe the fast microstructural changes at high temperatures where atomic diffusion is rapid. Therefore, the dislocation during the formation of austenite from martensite has not been clarified.

In this study, an originally designed ultrafast heating and cooling system was installed at the world's most advanced X-ray free electron laser facility, SACLA. Structural changes in martensite at a heating rate higher than 10,000 °C/s were observed by femtosecond X-ray diffraction. The instantaneous dislocation during the ultrafast heating of the martensite structure in steel was quantitatively observed for the first time in the world (Fig. 1). The microstructure formation process was clarified from the perspectives of dislocation density and carbon concentration (Fig. 2). The *in situ* observation of dislocation and carbon concentration related to microstructure formation in steel will lead to improvements in the performance and quality of steel, the development of new alloys or new manufacturing processes, and other major breakthroughs.

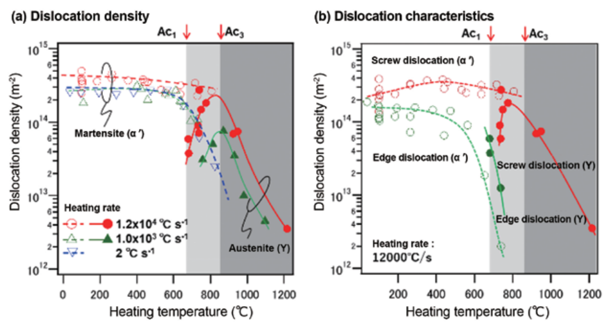


Fig. 1 Changes in dislocation density and dislocation characteristics during heating.

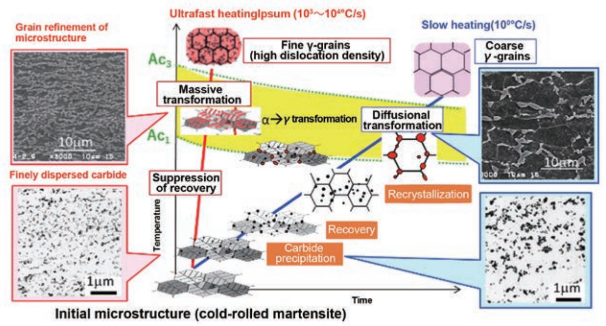


Fig. 2 Structural changes during ultrafast heating.

SACLA BL3 Mitsuharu Yonemura (Nippon Steel Corporation)

Article: M. Yonemura *et al.*, *Scientific Reports* **9**, 11241 (2019).