Femtosecond single-shot imaging with MAXIC at SACLA

X-ray Free-Electron Lasers (XFELs) are a new generation of X-ray sources that produce ultrafast (~10^{-14} s) and ultra bright (~10^{12} photons per pulse) coherent X-ray pulses that allow for unique and innovative experimentation. However, in contrast to synchrotrons, where multiple end stations are available, XFELs are single user facilities. Given the current limited availability of XFEL sources, experimental platforms at these facilities should be adaptable, accommodating both novel and routinely requested experimental set-ups. To this end we have developed the Multiple Application X-ray Imaging Chamber (MAXIC), a generic platform for performing a wide variety of single-shot, coherent diffraction experiments at SACLA [1]. Here the current status of the chamber will be outlined and recent successful experiments using the MAXIC will be highlighted [2].

The MAXIC is currently available for installation in EH3 of BL3 at SACLA, as shown in Fig. 1. The main chamber is compact, with several flanges and ports compatible with beamline components such as; a high power optical laser, Multiport CCD (MPCCD) detector and focusing mirrors [1]. Inside the chamber are several high precision XYZ motor stages (Khozu Precision Co.) for alignment of samples, optical and pulse characterization instrumentation such as a set of 4-way cross slits, a Ce:YAG screen and a 50 μm gold cross wire.

XFELs provide a means to overcome traditional radiation damage barriers in diffraction experiments. Their high peak brilliance and short pulse duration allow snapshot diffraction patterns to be captured from samples faster than radiation induced damage can propagate [3]. This requires a high sample renewal rate, delivering a fresh sample to each pulse. The MAXIC can facilitate several single-particle delivery schemes outlined in Figs. 1(b-d), categorized as either “fixed” or “flying” [1]. “Fixed” targets are generally randomly dispersed onto thin, multi-windowed Si_3N_4 membranes. Raster scanning across the XFEL focal point, whilst synchronizing with a fast shutter, ensures a single exposure at each scan position. “Flying” samples are delivered to the XFEL focal spot in a continuous particle stream. They may be delivered in a gas focused liquid jet, ideal for serial crystallography, or in the gas-phase via an aerodynamic lens stack (ADL), preferable where low background noise is essential.

The true test of an experimental platform, however, is in its application to novel scientific problems. We have employed the MAXIC in experiments probing the nano-scale structure of a self-assembling, micron-sized macromolecule, the RNA interference (RNAi) microsponge [4]. The microsponges are composed entirely of long RNA strands and each strand contains multiple copies of a short interfering RNA (siRNA) sequence, which can be cleaved from the sponge to enter the RNAi pathway and alter expression of target genes [4]. Previously published work focused predominantly on the surface morphology of the microsponges, however for a more detailed understanding of its structure and function, characterization of internal morphology is also necessary. We applied the Coherent Diffractive Imaging (CDI) technique in this study as it allows quantitative views of electron density variation in micron thick samples without the need for sectioning or labeling [5].

CDI experiments were performed at beamlines **BL3** of SACLA and **BL29XU** of SPring-8, the results of which are shown in Fig. 2. RNAi microsponges

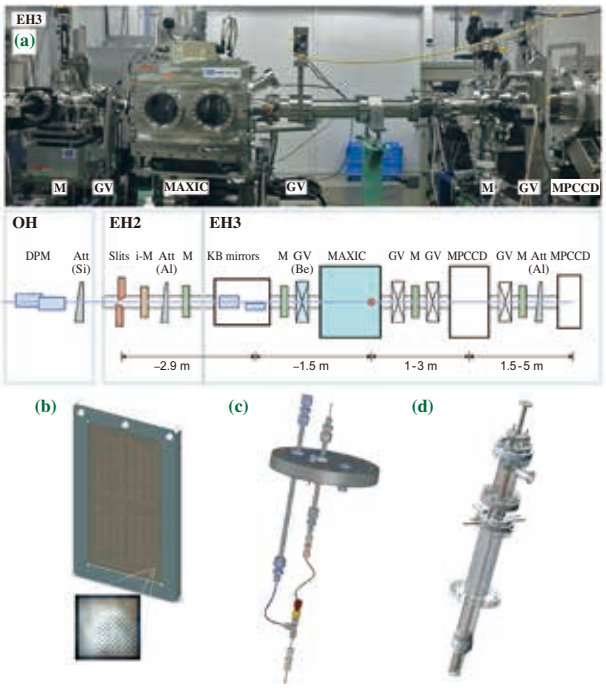

Fig. 1. Installation of the MAXIC at BL3 and key components. **(a)** Photograph of the MAXIC installed at EH3 of BL3 (top) and the overview of total layout within BL3 (bottom). M and GV represent beam monitor and gate valve positions respectively. Red circle represents the XFEL focal spot. **(b)** Fixed target sample holder with two multi-window Si_3N_4 membranes attached and a photograph of a single membrane after exposure to XFEL pulses (inset). **(c)** Flexible liquid jet injector. **(d)** Gas-phase injector with ADL stack.

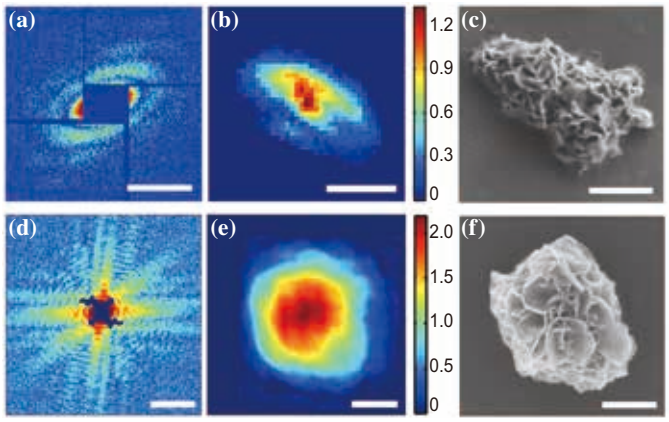

Fig. 2. Coherent diffraction imaging experiments at SACLA and SPring-8. **(a)** Single-shot diffraction pattern of an isolate microsponge. **(b)** Reconstructed image. **(c)** SEM Electron micrograph of a similar microsponge. **(d)** Averaged diffraction pattern of an isolate microsponge. **(e)** Reconstructed image. **(f)** SEM Electron micrograph of a similar microsponge. Color map represents relative electron density. Scale bars represent 10 μm^{-1} (**a** and **d**) 250 nm **(b)** and 500 nm (**c**, **e** and **f**).

were prepared according to published methods [4] and dispersed upon multi-window Si_3N_4 membranes. These were then mounted either in the MAXIC or a synchrotron based CDI chamber. For single-shot experiments samples were exposed to 2 μm focused, 5.5 keV pulses from SACLA. A far-field diffraction pattern from an individual microsponge was captured (Fig. 2(a)) and its electron density reconstructed via iterative phase retrieval algorithms (Fig. 2(b)). The image shows a progressive change in internal density from a high density central region. This conforms well with images reconstructed from full-sized microsponges analyzed at SPring-8 (Figs. 2(d-e)).

To gain greater insight into these internal features Coherent Diffraction Tomography (CDT) was performed at BL29XU. Here diffraction patterns are collected over a ±69° tilt series and combined to reconstruct a complete 3D electron density. The results in figure 3 display a distinct high density "core-like" region buried within the microsponge. The average electron density of this region is 0.54 e^- per $Å^3$, close to the nominal electron density of RNA of 0.55 e^- per $Å^3$, suggesting that it is composed almost exclusively of densely packed RNA strands [2]. To further confirm this feature coherent SAXS (cSAXS) experiments were performed at SACLA. A sample cSAXS pattern, captured from a cluster of RNAi microsponges, is shown in Fig. 3(b). The pattern was radially averaged to produce a 1D curve and then this was fit to a simple core-shell ellipsoid model, based upon observations from CDI, as shown in Fig. 3(c). The model shows a consistent fit to the experimental data, with a normalized r.m.s. error of 0.8%, suggesting that the core-shell morphology is a generic feature of the RNAi microsponge [2].

In summary we have developed a novel platform for a variety of coherent X-ray diffraction experiments at SACLA. We have demonstrated an analysis scheme for combining single-shot experiments at an XFEL with more conventional CDI experiments at a synchrotron. We have applied this scheme to uncover the core-shell morphology of a novel biomolecular construct, the RNAi microsponge.

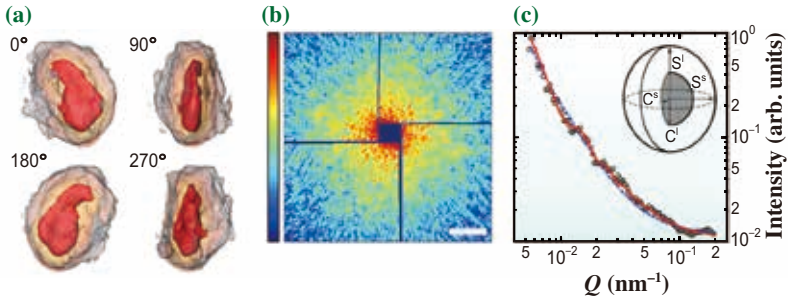

Fig. 3. Coherent diffractive tomography and cSAXS. **(a)** Reconstructed 3D electron density at various rotation angles relative to the y axis. **(b)** Sample cSAXS pattern. Color map represents relative intensity. **(c)** 1D scattering curve fit to a simple ellipsoid model (blue dashed line) and core-shell ellipsoid model (red line, model inset).

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