Challenges regarding the 3D imaging of biological macromolecules in the gas phase -
Preparing for x-ray free electron laser experiments

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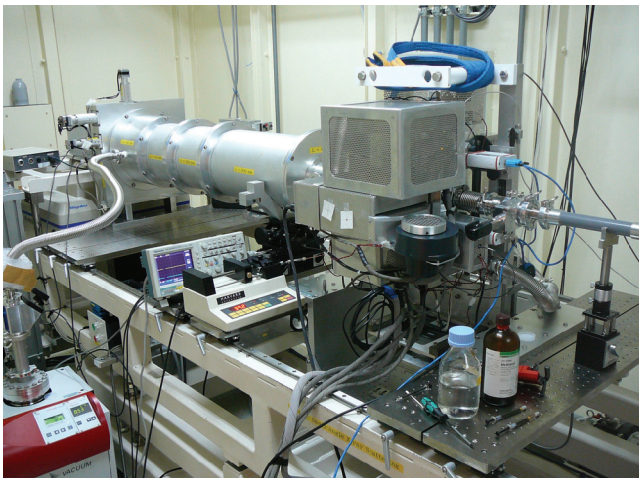
Synchrotron radiation has had a major influence on structural biology, including the structural characterization of a wide variety of proteins and their complexes. New science opportunities are emerging with the exploitation of x-ray free electron lasers. One of the 'grand challenges' of structural biology in the years to come is to successfully image isolated biological molecules, without the need of a crystalline sample [1]. Among the many experimental challenges for these kinds of experiments are the manipulation and delivery of macromolecules in well-defined state for x-ray scattering/diffraction experiments *in vacuo* in the interaction region. These areas need to be developed and optimized to achieve sufficient particle densities and improve sample selection so that interaction between x-ray laser pulses and macromolecules is produced and recorded efficiently. Small-angle x-ray scattering is a technique for the 3D conformational analysis of a variety of materials including biological macromolecules [2]. As no crystals are involved, the method provides an ideal means to investigate multi-domain proteins and protein complexes that are hard to crystallize. For the structural analysis of proteins and their complexes, the interaction between sample and x-rays is generally performed when the proteins are present in solution. A 3D digital ion trap was developed as sample delivery system that can be interfaced with a high intensity x-ray source [3] so that experiments can be performed on ions isolated in the gas-phase. In preparation for XFEL studies synchrotron x-ray scattering experiments with this sample environment have been performed at SPring-8 in collaboration with scientists from the RIKEN SPring-8 Center. The encountered challenges will be reported. The full integration of the mass spectrometer into the beamline BL45XU (incl. the removal of instrument isolating windows, see figure on left), almost maximum synchrotron photon flux available and an x-ray counting detector with essentially zero background



provided clear improvements over previous synchrotron experiments. It is now apparent that continuous synchrotron x-ray measurements are required with the highest possible ion population in the 3D trap over long time periods (of the order of tens of minutes up to hours) concomitant with the lowest background signal from instrument and beam characteristics. Yet, these photon-hungry experiments are expected to transform with an XFEL beam where one estimates that a few ions would be intersected by a single x-ray laser pulse of some tens of femtoseconds.

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