ESRF	**Experiment title:** **Structural dynamics of working proteins -** **F1ATPase, ATP-synthase and chaperonines GroEL, GroES**	**Experiment number**: LS722 (& LS717) (cooperation)
Beamline: ID02 / BL4	**Date of experiment**: from: 3.9.1997 to: 9.9.1997	**Date of report**: 28.1.1998
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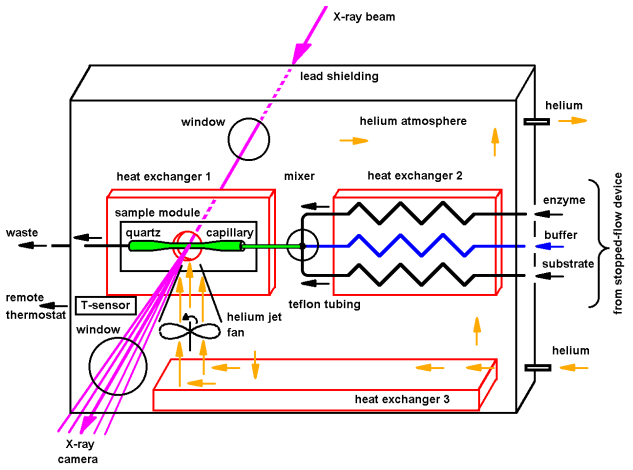
Report:

Conclusion:

The experimental sessions LS722 & LS717 (collaboration projects) were successful in investigation of working proteins during the biological reaction (F1ATPase, ATP-synthase, GroEL) with the full flux of the ID02 beamline using a CCD camera:

- The XRII CCD-camera withstands the full flux of the beamline (10^{13} photons/s at sample). A camera burn-out, as observed with a 2D-gas detector (test 05/1997) was absent.
- The signal was highly stable (after 1 d $<10^{-3}$), which enables automatic evaluation.
- A novel helium-jet cooled sample environment was constructed. This allowed temperature control even with 10 mW absorbed power and precise buffer subtraction (error $<10^{-3}$).
- The enzymes withstand the full radiation (10^{13} ph/s at 12 keV) for a time sufficient for estimation of a structural film (>40 s) in the presence of a radical scavenger and de-gasing
- A computer controlled stepper motor driven double-stopped-flow device enabled start and synchronization of the enzymatic reactions by a concentration jump after rapid mixing. The results of the successful experiments with F1ATPase/ATP-synthase and GroEL are described in separate experiment reports (LS722, LS717).
- The unexpectedly short duration of the structure pulses of F1ATPase and ATP-synthase (<400 ms; 3% of cycle) produced a problem with the read-out blind phases of the CCD-camera. This effect is known as "aliasing" and produces an extra-error. This will be abolished by an improved camera read-out device with 4 fibre lines (50 ms resolution).



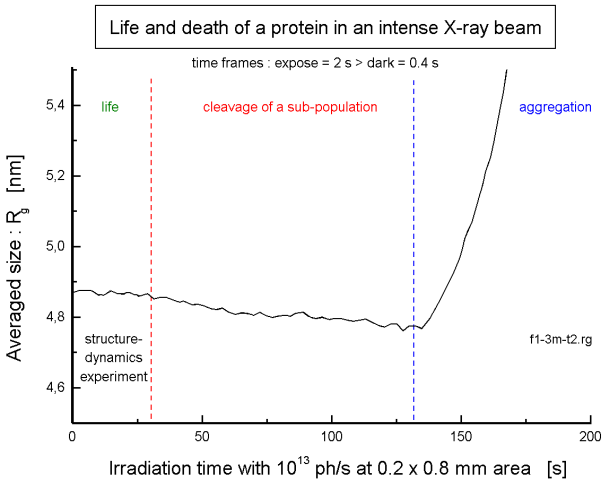


Fig.1: Helium-jet cooled sample environment **Fig.2:** The life time enables a structural film

As improvement to the experiment "test 05/1997" at the ID02 beamline a XRII-CCD camera (P. Labiche) was used in the LS722 experiment instead of a 2D-gas detector. This showed no radiation burn-out effect, even with the full flux and strong scattering of a concentrated protein solution. At a sample-detector distance of d = 1m we observed 100,000,000 scattered photons/s with the full flux of the beamline, i.e. 10^{13} ph/s (12 keV) on 0.2 x 0.8 mm. The signal was stable for at least 2h under full beam and highly reproducible ($<10^{-3}$ after 1d).
We have developped a novel sample environment for the investigation of fluids at high flux synchrotrons shown in Fig.1. The sample is irradiated in a flow-through quartz capillary (1.3 mm diameter) on one tree of a three-circuit heat exchanger with a sensor for remote control of a thermostat. The capillary is cooled by a helium-jet. The 3 components are supplied by a novel computer controlled stepping motor driven double-stopped-flow device. In the LS722 / LS717 experiments we mixed for each shot 2 x 70 µl of enzyme solution (1% protein) with CaATP-buffer (2 mM). The second drive was used for flushing with protein-free buffer.
The signal precision obtained with the helium-jet cooled sample environment, stopped-flow, and CCD camera was so high that an on-line evaluation of the data is possible. This can be usefull in kinetic studies, as those structural films may consist of some 100 GB camera data if the reaction is investigated repetitively at several time resolutions and temperatures.
We tested scattering behaviour and radiation stability of buffer, test proteins (BSA, mBR monomeric bacteriorhodopsin) and object proteins (F_1ATPase, ATP-synthase, GroEL, GroES) with 1% solutions. The very first results in the flux range of 10^{13}-10^{14} ph/s*mm^2 are:
- gas bubble production is avoided by de-gasing of the samples.
- the temperature is stable (1°, >5 min.) with the full beam (10 mW abs.) using the helium jet.
- the protein radiation stability was improved by a radical scavenger (10% v/v glycerol, i.e. a 1000 fold molar excess to the protein) and by removal of oxygen by de-gasing.
- the radiation stability of proteins was >40s detected by time resolved small angle scattering.
- for long time experiments (1 min ...2 h, protein-assembly) we used an intermitting beam.

As shown in Fig.2 the life time (>40s) of native F1ATPase (de-gased sample, glycerol) is long enough for the estimation of a structural film of the working protein (the reaction cycle is finished after < 20 s). This is the time window for structural biology of working proteins.