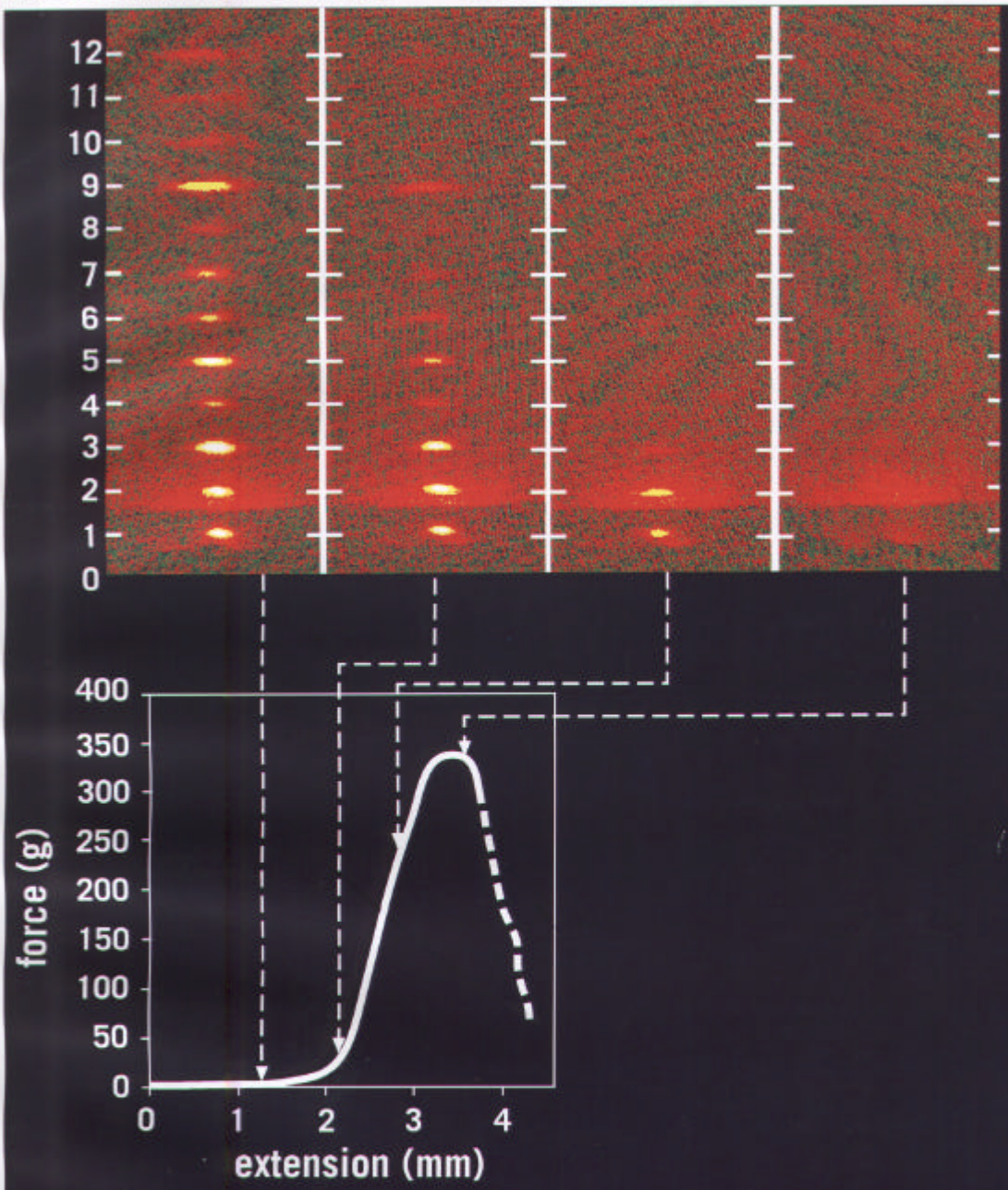
AUSTRIAN SAXS - BEAMLINE AT ELETTRA



COLLAGEN STRUCTURE UNDER TENSION

ANNUAL REPORT 1996/1997

AUSTRO - SAXS

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ELETTRA - SAXS beamline yearly report 1997

Time resolved investigation of protein dynamics - working native F_1ATPase

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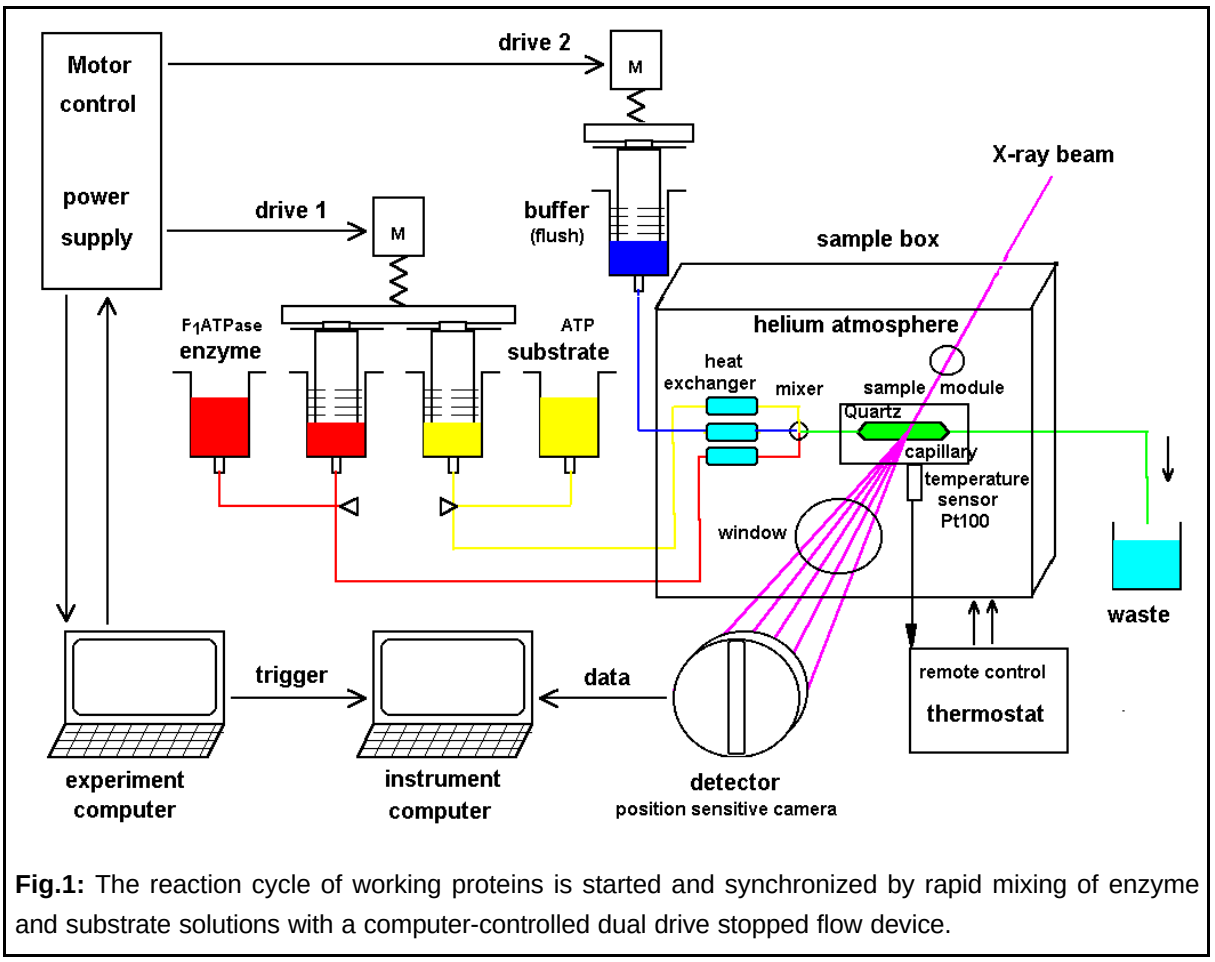

Fig.1: The reaction cycle of working proteins is started and synchronized by rapid mixing of enzyme and substrate solutions with a computer-controlled dual drive stopped flow device.

The reaction cycle and the molecular regulation of several large multidomain proteins depends on structural changes of the protein complex. This is suggested indirectly by enzyme kinetics, thermodynamic studies and time resolved spectroscopic studies of enzymes, e.g. bacteriorhodopsin, photosynthetic proteins, cytochrome oxidase and ATP-synthase or its catalytic F1-fragment (F_1ATPase) [1]. In some cases the protein structures have been estimated before and after the enzymatic catalysis by X-ray crystallography, e.g. with the proteins hexokinase, ras and EF-Tu. Nevertheless the online analysis of structural dynamics during protein activity requires the unrestricted flexibility and accessibility of the protein to diffusion of substrate and products. This is in most cases only possible with protein solutions, which can be structurally investigated by time resolved small angle scattering.

At the SAXS beamline of ELETTRA a novel experimental setup for the investigation of transient structural changes of working protein solutions was installed. As shown in Fig.1, the setup consists of 3 devices: i) a computer controlled stepping motor driven dual drive rapid mixing device (stopped flow), which delivered enzyme, substrate and flush-buffer solutions at selectable volume, speed and repetition rate; ii) a sample environment box with a flow-through quartz capillary (1.1 mm diameter), tefzel mixer,

3-branched heat exchanger, Pt100 temperature sensor and helium atmosphere; and iii) a remote control thermostat connected to the sensor at the sample module inside the box. After the first experiment series the box was additionally equipped with a helium jet fan blowing on the irradiation point at the capillary and then used for experiments at ELETTRA and ESRF. The beam heating was less then 0.2 K/min with 2 x 10^{12} photons/s (8 keV) in an irradiated area of 0.8 x 1.5 mm (negligible). The radiation demage of the proteins was suppressed by de-gasing (removal of oxygen) and addition of a radical scavenger (10 % glycerol) in 1000 fold molar excess with respect to the protein concentration (5g/l, i.e. 10 µMol/l with ATP-synthase; M=500.000).

Due to the high stability and reproducibility of the sample geometry, the experiments were each done as a **series of four subexperiments** with scattering of: **1)** protein solution I_{ps}; **2)** solvent I_s; **3)** empty dry cell I_e; and **4)** dark (intransparent) sample I_d. As dark sample we used saturated RbBr solution, which is the equivalent to the Cd-probe in neutron scattering for X-rays. In the evaluation the contribution I_d was subtracted from each of I_{ps}, I_s, and I_e after normalization to the incoming beam monitor $\mathbf{B} = \int \mathbf{I(t)\,dt}$, whereas the scattering contribution of the protein I_p was estimated by subtraction of the components after additional normalization with the sample transmission T, estimated with a pin diode:

$$\mathbf{I_p(q) = [(I_{ps}/B_{ps} - I_d/B_d)/T_{ps} - (I_e/B_e - I_d/B_d)/T_e] - [(I_s/B_s - I_d/B_d)/T_s - (I_e/B_e - I_d/B_d)/T_e]}$$

When protein and solvent experiments were done within one day, the empty cell correction was ommited. The scattering vector (momentum transfer) was expressed as $\mathbf{q = (4\pi/\lambda) \cdot \sin\theta}$ where θ is the half scattering vector.

ATP-synthase is a membrane protein of cell energy metabolism, that transfers the energy of respiration by synthesizing the energy rich chemical compound ATP to other biologic systems, e.g muscle [1].

In Fig.2 the X-ray small angle scattering of the catalytic head fragment F_1ATPase (10 g/l) obtained at the SAXS beamline in 1000s with an 1-dimensional gas-detector is shown as Guinier representation. The plot yields the averaged size of the molecule of R_g = 45.8 ± 0.28 Å, which is suggested to change during the catalytic reaction [2] as well as upon the molecular regulation [3]. The result fits well the properties obtained with long-time scattering experiments at the DESY synchrotron obtained in 4x3 h with a 2D-camera [4]. Stopped flow experiments showed that an acceptable error (ΔR_g < 1Å) is obained with the 1d-camera in 20 shots of 2 s time resolution. Thus we have started the investigation of the interaction of the catalytic sites by comparison of azide-inhibited [5] and native F_1ATPase and ATP-synthase, where we see a 3-fold expansion of the molecule during action.

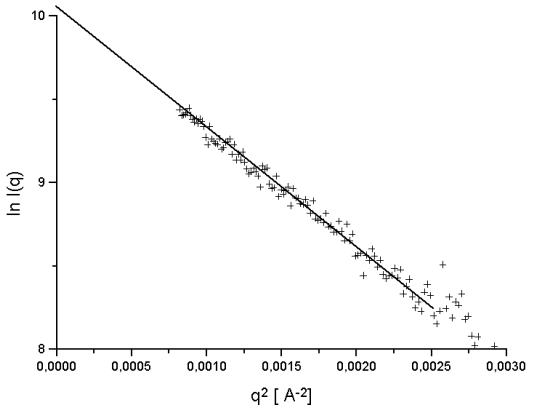

Fig.2: X-ray small angle scattering of native F_1ATPase (10g/l) as Guinier representation, which yields the average size of the molecule of R_g = 45.8 ± 0.28 Å.

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