

5.2 Temperature-jump

5.2.1 Electrical-discharge-induced T-jump

5.2.1.1 *T-jump apparatus*

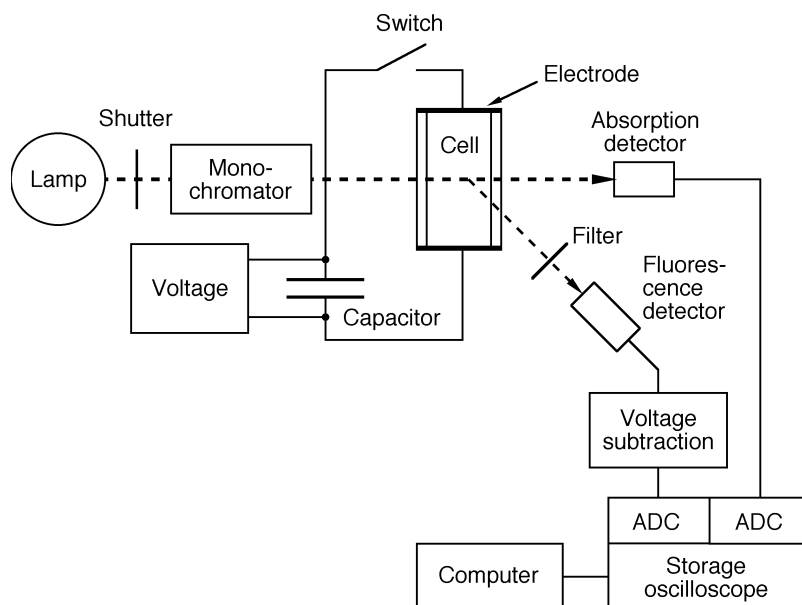


Fig. 5.6. Electrical-discharge-induced temperature-jump (T-jump) method. A capacitor is charged by a power supply up to a specific voltage and then rapidly discharged through the sample cell that contains the protein in a buffer with a certain electrical conductivity, for example, 50 mM phosphate buffer with 100 mM KCl. The electrical discharge causes Joule heating by 1–20°C with rise times of typically 500 ns – 10 μ s, depending on the instrument settings, in particular on resistance of the protein solution and capacitance. When starting from the (partially) cold-unfolded state, increase of temperature causes refolding, otherwise fast unfolding reactions may be studied. The reaction kinetics is followed by absorption or fluorescence detection. The electrical signals are digitized by analog-to-digital converters (ADC) which are part of an Nicolet (Madison, WI) model Pro 90 storage oscilloscope and further processed on a computer. Large sample cells with 1 mL volume may be used which enables a high light throughput and thus an excellent signal-to-noise-ratio (see Fig. 5.8). Minimization of photolysis in the sample by the intense light of a 200-W mercury–xenon lamp is achieved with the help of an optical shutter that opens only during the measurement. Prior to digitalization of the fluorescence signal by an ADC, a constant voltage is subtracted (see Fig. 5.9). Therefore, a 12-bit ADC is usually sufficient to resolve changes of only 0.01% in the nanosecond time scale. When using a well-stabilized power supply for the lamp, usually no reference channel is needed. Otherwise the signal of a reference detector that is located between monochromator and sample cell (not shown) may be used. The simplicity of the device that does not contain mechanically moving parts makes the handling very easy and causes an exquisite reproducibility.

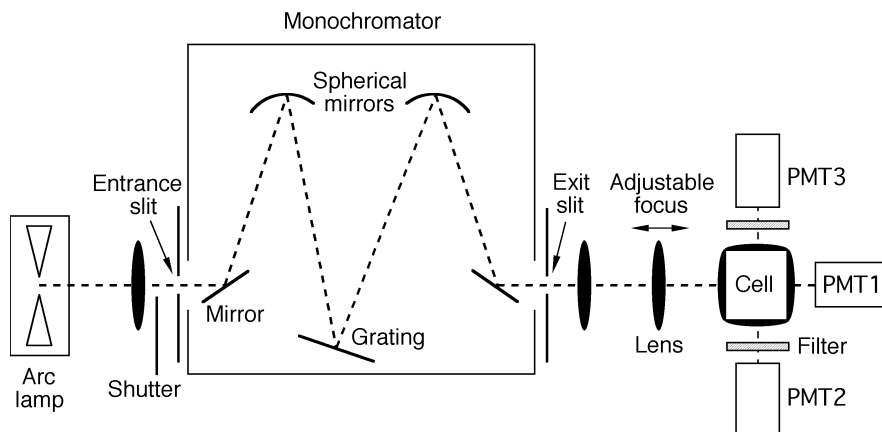


Fig. 5.7. Optics of an electrical-discharge-induced T-jump apparatus (Fig. 5.6). Photomultiplier tube 1 (PMT1) is used for absorption measurements. For fluorescence detection, the electrical signals of photomultiplier tubes 2 and 3 (PMT2 and PMT3) are added. To decrease the photon shot noise, the optics is optimized for a large light throughput (e.g., relatively wide bandwidths of fluorescence excitation and emission are used for the experiments presented in Chap. 10) and a high aperture of fluorescence detection (DIA-LOG, Düsseldorf, Germany; Eigen and deMaeyer, 1963; French and Hammes, 1969; Nölting et al., 1995, 1997a).

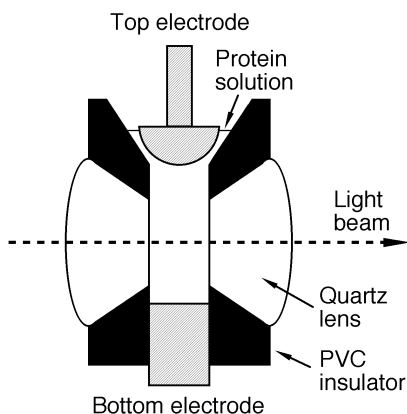


Fig. 5.8. Sample cell for an electrical discharge T-jump apparatus. In order to avoid pressure due to thermal expansion upon T-jump, the top of the cell is not sealed. Fluorescence detection is perpendicular to the excitation beam (DIA-LOG, Düsseldorf, Germany; Eigen and deMaeyer, 1963; French and Hammes, 1969; Nölting et al., 1995, 1997a).

Protein solutions that contain electrolytes may have sufficient electrical conductivity to enable them to be heated by a rapid electrical discharge through the sample cell (Figs. 5.6–5.10). With the simple design (Eigen and deMaeyer, 1963; French and Hammes, 1969) Joule heating with rise times of $\approx 1 \mu\text{s}$ or faster can easily be achieved when using a buffer with 100 mM KCl. The large size of the sample cell of about 1 mL volume enables a large light flow and thereby leads to a low photon shot noise. Noise levels of $<0.01\%$ root mean square (rms) of the fluorescence signal have been achieved at a 5- μs response time of the electronics (Nölting et al., 1995). The amplitude of temperature-jump (T-jump), ΔT , is given by

Protein Folding Kinetics

Biophysical Methods

Nölting, B.

2006, XVI, 222 p. 170 illus., 12 illus. in color., Hardcover

ISBN: 978-3-540-27277-9