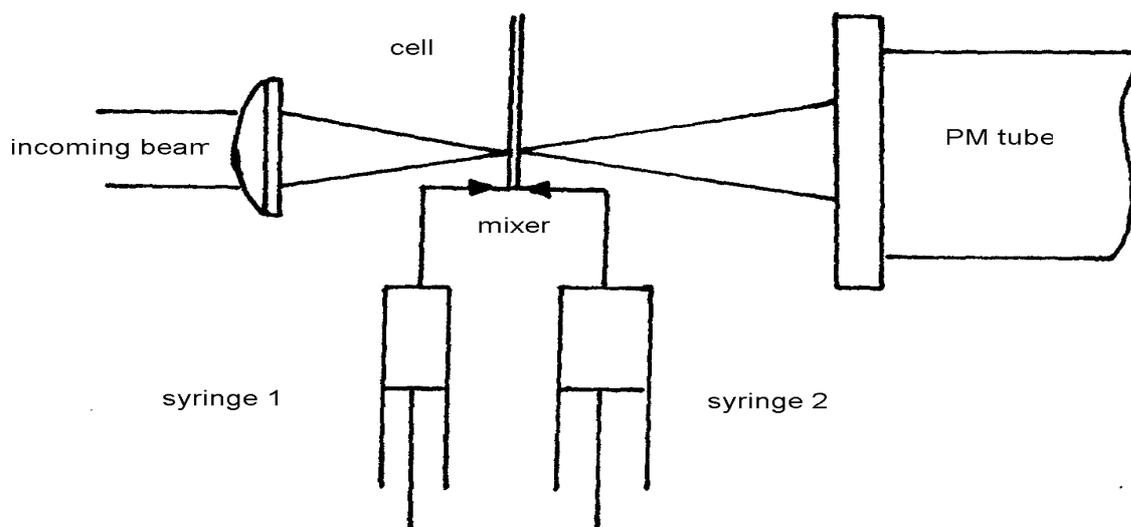


RAPID MIXING CD EXPERIMENTS

A very interesting paper has just been published [*Nature Struct. Biol.*, 7, 514-520 (2000)].

Prof Morishima and co-workers are showing stepwise formation of α -helices during cytochrome c folding in submillisecond time frame. They were using a conventional CD apparatus (Jasco J-720) coupled with a continuous flow mixer of their own design¹.



Solutions (denatured protein and refolding buffer) are continuously pumped by two syringes into a μ mixer and a thin observation cell (250x250 μ m) is closely connected to the mixer.

Time resolved spectra measurement (at reasonable scanning speeds) are possible changing either the flow rate or (as in this case) the distance between the observation point and the mixer.

Authors reported spectral deformations coming from mechanical strain of the μ cell² (and to me also from the necessary lens to focus the observation beam). Other potential source of artefact is coming (not in this case, but with other samples) from the LD (linear dichroism) of the sample, since the system operates here with very high shear rate (see our Technical Report N° 16 of June 2000).

Can we forecast a commercial accessory?

Probably yes, while I was not yet aware of Prof Morishima article, Yves Dupont from CEA-Grenoble and *guru* of Bio-Logic (dupont@dvsud.cea.fr) sent me preliminary information on the Bio-Logic microsecond mixing system they are developing for their SFM stopped flow modules, which can be easily operated in continuous flow mode. Different ageing time in collecting spectra will be possible changing flow rate of the syringes (stepping motor controlled) and/or by a micrometric stage changing the position of the full assy (syringes/mixer/observation cell) in respect to the beam.

The collimated light-path of the Jasco J-810 allows an easy interfacing, even if for a more punctual sampling it'd be convenient to reduce the monochromator slit height.

Let's see what the future will bring us, this article is really showing something new and exciting.

¹ Takahashi S. et al, *Nature Struct. Biol.*, 4, 44-50 (1997)

² Takahashi S. et al. *Old and new views of protein folding* (eds. Kuwayima K. & Arai. M.) 75-84 (Elsevier Science, Amsterdam; 1999)