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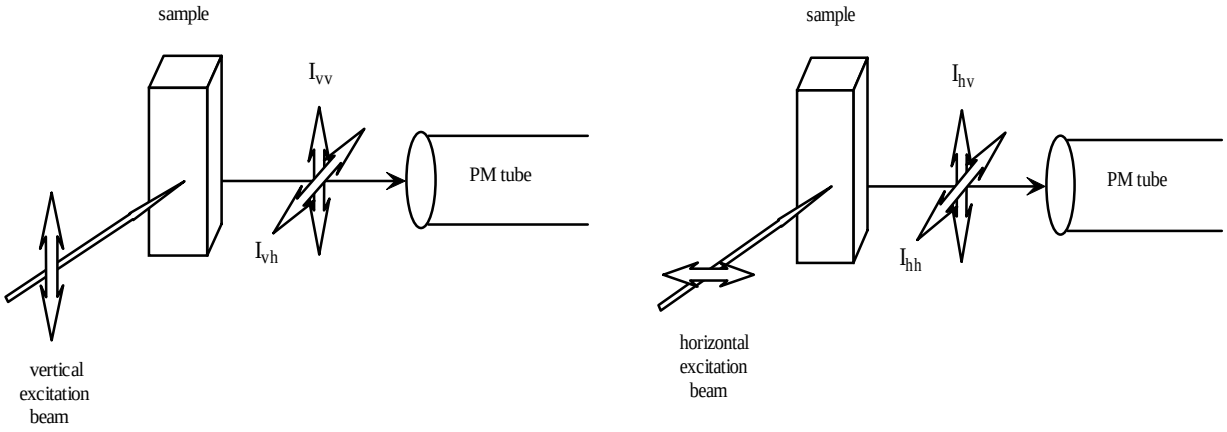
FLUORESCENCE ANISOTROPY

In a previous T.R. (N° 14, May 2000) same argument was shortly discussed. Let's go a bit deeper this time.
A recent article[1] illustrates how stopped-flow fluorescence anisotropy may be a valuable tool to study protein folding.
Basically any CD spectrometer equipped with 90° detection and dual frequency lock-in detection may be used for the application.
Following text of the article, let's suppose we alter the excitation polarization between vertical and horizontal (using our PEM in half-wave mode):



Anisotropy can be calculated as follows[2]:

$R = (I_{vv} - G \times I_{vh})/(I_{vv} + 2 \times G \times I_{vh})$

In our set-up G (Ihv/Ihh) can be assumed equal to one, since we detect the fluorescence without any polarizing sensitive device (i.e. a simple photomultiplier tube with a filter behind*)
* *in case a monochromator is used in the emission path G must be measured, alternatively a depolarizer may be inserted in the path or a fiber bundle may be used in the transfer to act effectively as a depolarizer*

Changing the linear polarization of the incoming beam through the piezoelastic modulator we assure also that the intensity of the incoming beam is constant.
Our detector will measure:

$A_v = I_{vh} + I_{vv}$ when polarization is vertical

$A_h = I_{hh} + I_{hv}$ when polarization is horizontal

Due to 90° mount $I_{vh} = I_{hh} = I_{hv}$, so

$A_h = 2 \times I_{vh}$

So anisotropy **$R = (A_v - A_h)/(A_v + 0.5 \times A_h)$**

[1] Canet. D., Doering. K., Dobson C. M., Dupont Y., *Bioph. Journ.*, 80, 2001, 1996
[2] Lakowicz J.R., *Fluorescence Anisotropy*, in Principles of Fluorescence Spectroscopy, J.R. Lakowicz ed., Kluwer Academic/Plenum Publisher, New York, 1999, 291

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The advantages of the approach versus the use of a normal fluorometer equipped with polarizers are mainly: speed and sensitivity.

The technique has been patented in France by CEA (Commissariat Energie Atomique) and extension to other countries will take place. Following this idea Bio-Logic, the French stopped-flow unit manufacturer, prepared a specific kit to modify existing Jasco J-810 (but it'd work as well on any other earlier Jasco CD) to measure steady state and mainly stopped-flow anisotropy. The kit* includes:

- photomultiplier detector and lock-in amplifier PMS400 & PMT (ref 048-20)
- connection box for PCI-6052E (ref 048-29)
- acquisition board PCI-6052E & cable (ref 083-11)
- Bio-Kine 32 software (ref 083-02)**

The Jasco J must be simply modified to extract PEM ref signal and PEM driver must be tuned to operate as half-wave retarder (this facility is standard in any Jasco modern CD apparatus, but proper tuning is necessary when you install the kit the first time).
The A_v and A_h components are collected from the PMS400 and the Bio-Kine will compute both anisotropy and regular fluorescence.

** the kit fits the Bio-Logic SFM, for steady state single wavelength or melting experiments you must (easily) adapt the PM tube on the 90° port of the J or fit the SFM with Peltier cell holder …. In any case no spectra mode is presently possible*

*** Bio-Kine is also able to control the Jasco J-810 in fixed wavelength modes (not previous models)*

With the above configuration you are able to perform CD and anisotropy stopped-flow experiments (with the Bio-Logic SFM of your choice). The paper listed above clearly indicates how methods can be well complement each other, with the clear advantage of anisotropy/S.F. versus CD/S.F. that the sample concentration can be far smaller.

The alternative to the Bio-Logic hardware is to equip your unit with a standard LD (linear dichroism) accessory (LD-403).
In this case, unless you extract A_v and A_h components (which for an hardware modification is necessary) you can measure alternatively an AC signal (at 100 kHz = twice the PEM frequency) and a DC signal, where:

$$\mathbf{AC = A_v - A_h} \quad \text{and} \quad \mathbf{DC = (A_v + A_h)/2}$$

Both these signals are available from the LD-403 board, and you must feed with the AC an external signal port. Signal must be further adjusted (simple hardware modification) to keep on the board same gain for both AC and DC components*.

** as a matter of fact you cannot collect the data in the LD mode since standard Jasco software performs in this mode a log conversion for ΔOD display.*

The plus of this approach are possibility to use also in spectral mode and the fact that no external box/wiring is necessary.
So anisotropy can be easily calculated by post run data processing.

$$\mathbf{R = (A_v - A_h)/(A_v + 0.5 \times A_h) = AC/(1.5 \times DC + 0.25 \times AC)}$$

Further tests with the Jasco LD-403 board will be carried on in cooperation with Mr Dupont of CEA-Genoble.

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