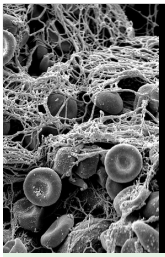

Linear Dichroism: the study of fibrin and other biological fibres

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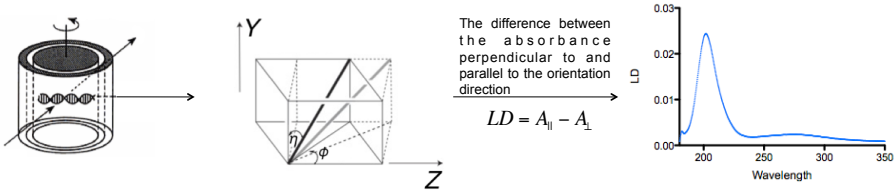
I. Introduction: what is linear dichroism?

Linear dichroism (*LD*) is a spectroscopic technique measuring the photons absorbed by an aligned sample from different planes of polarised light, to determine the structure and orientation [1]. To obtain an *LD* spectrum the molecules in a sample are first aligned by shear force as the solution is passed between two concentric sleeves. The sample is then irradiated with linearly polarised light in two planes, and the absorbance measured.



Couette flow aligning

Straight line arrows represent the two planes to which linearly polarised light will be applied once alignment is achieved

Molecular movement in shear flow

As molecules align they rotate toward the *Z* axis (the orientation direction). The black line represents a starting orientation, the grey line the progression after δt of shear force (G)

An *LD* spectrum is plotted

An example *LD* response of collagen fibres. The peak at 202 nm is a result of π-π^* transitions of the peptide backbone

Figure 1. Schematic for the experimental process of obtaining an *LD* spectrum

II. Stopped-flow *LD*

Using stopped-flow *LD* it is possible to study the behaviour of molecules as they align and relax by Couette flow.

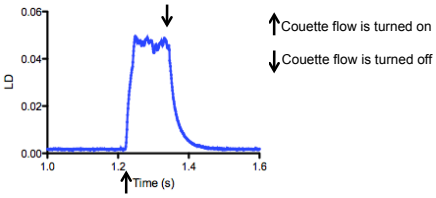


Figure 2. Stopped-flow *LD* of fibrous DNA with varying solution viscosities: 1.33cP (blue), 1.44cP (red), 1.48cP (pink) and 1.69cP (black).

We derive a model to describe the behaviour of molecules as they align under shear flow using differential equations for the angles η and Φ (Figure 1) between the molecules in a sample and the orientation axis

$$\frac{d\eta}{dt} = G\sin\eta\cos\eta\cos\phi\sin\phi \qquad \frac{d\phi}{dt} = G\cos\phi^2$$

The alignment of molecules is poorly modelled by this current theory. Figure 3 demonstrates the effect of altering the viscosity in failed attempts to better fit the data.

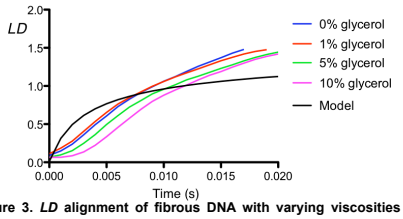


Figure 3. *LD* alignment of fibrous DNA with varying viscosities and model response (black)

III. Modelling the relaxation of molecules

In comparison, the relaxation of molecules after shear flow alignment has been well modelled [2]

$$LD = \frac{1}{a + bT^c}$$

where ***T*** is time, ***a, b*** and ***c*** are fitted variables where ***c*** is a parameter describing the number of relaxation processes occurring. ***c=2*** *for* a single relaxation process, ***c<2*** *for* a distribution of processes. Figure 4 shows the experimental data and the model predicted response for DNA fibre relaxation. The data is well described by the model, fitted values for ***c*** propose that fibre relaxation is complex, with a distribution of processes.

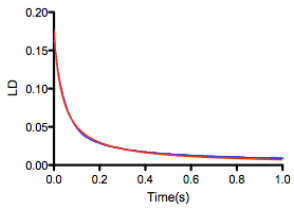


Figure 4. Decay in orientation of DNA fibres. Experimental data (blue), model fitted response (red)

IV. Interaction between fibres

The stopped-flow *LD* for the relaxation of fibres can be applied to study fibrin fibre aggregation. Here we have studied the relaxation of collagen and peptide fibres to demonstrate fibre interaction during alignment

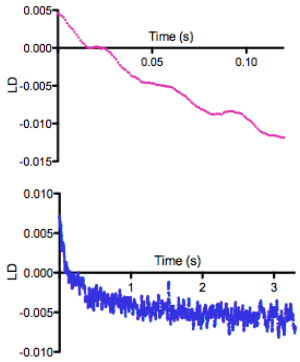


Figure 6. Orientational relaxation periods of short peptide fibres (pink) and collagen fibres (blue)

The longer relaxation time for collagen suggests an aggregative interaction is occurring during alignment. We can use this same principle to study fibrin fibre behaviour.

V. *LD* of fibrin fibres

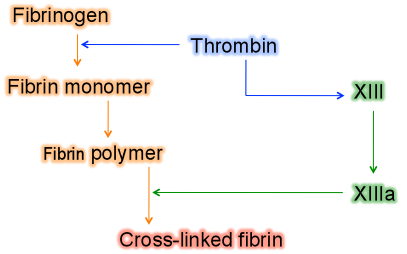


Figure 5. Conversion of fibrinogen to fibrin fibres with the enzyme Thrombin and human factor XIII

Our Couette flow system runs at a similar flow rate to that of the blood stream and is thus ideal for analysing fibrin. In the presence of thrombin, fibrin monomers are formed and are aligned under Couette flow. Polymerisation of the fibres can be monitored as an increase in the LD response.

However, if we are able to align and relax the monomers before they polymerise we can fit the experimental data and extract both the relaxation parameter, ***c*** and the relaxation time. These parameters could provide insight into the time dependence of fibrin fibre interactions.

VI. Conclusion

- *LD* is commonly used for structural identification, but less so for orientational modelling.
- Here we obtain stopped-flow *LD* data for DNA, collagen and peptide fibres, showing the *LD* response during molecular relaxation is well modelled.
- In the presence of thrombin, fibrinogen is converted to fibrin monomers which can be aligned under Couette flow
- Fitting the data to our models will afford further information regarding the interaction between the fibres

VII. References

(1) Norden, B. Rodger, A. Dafforn, T., Linear Dichroism and Circular Dichroism: A Textbook on Polarised-Light Spectroscopy. (2010)
(2) Champion J, Meeten G, Moon B. Flow turbidity in colloidal kaolinite dispersions. J. Chem. Soc., Faraday Trans (1978) 2, 1979, 75, 767-779

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