Methods to determine the pressure dependence of the molecular order parameter in (bio)macromolecular fibers (ESI)

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S1 The (molecular) order parameter of a grating of rotational symmetric fibers in parallel

Let a sample consists of a certain amount of fibres which are rotationally symmetric and oriented along the z direction. The absorbance values relative to the principle axes (A_{xx}, A_{yy}, and A_{zz}) are known. One is then able to calculate the three (molecular) order parameters corresponding to the principle axes using

$$S_{ii} = \frac{1}{2}\frac{2A_{ii} - A_{jj} - A_{kk}}{A_{ii} + A_{jj} + A_{kk}}, \tag{S1}$$

where the indices i, j, and k represent the principle axes x, y, and z, as well as, their cyclic permutations.

The grating of fibres can then be described as a convolution of a single fibre with a Dirac comb. In case of a single fibre, that provides rotational symmetry, it can be found that $A_{xx} = A_{yy} = A_\perp$ and $A_{zz} = A_\parallel$ (or $A_{xx} = A_{yy} = A_{min}$ and $A_{zz} = A_{max}$). As a result of rotational symmetry, S_{zz} is identical to

$$S = \frac{A_\parallel - A_\perp}{A_\parallel + 2A_\perp}. \tag{S2}$$

S2 Relation between dichroic ratio and order parameter in case of rotational symmetry

In case of rotational symmetry, one is able to calculate the dichroic ratio based on the molecular order parameter:

$$\begin{aligned} S &= \frac{A_\parallel - A_\perp}{A_\parallel + 2A_\perp} && \text{(S3)}\\ &= \frac{A_\parallel/A_\perp - 1}{A_\parallel/A_\perp + 2} && \text{(S4)}\\ &= \frac{D-1}{D+2} && \text{(S5)} \end{aligned}$$

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Consequently:

$$D = \frac{1+2S}{1-S} \tag{S6}$$

S3 Convolution of a Gaussian function with a discrete distribution

Let $f(\theta)$ be a normalized Gaussian distribution function with an arbitrary central moment θ_0 and standard deviation ω_0. Furthermore, let $g(\theta)$ be a normalized discrete distribution.

$$f(\theta) = \frac{1}{\sqrt{2\pi\omega_0^2}} \exp\left(-\frac{(\theta-\theta_0)^2}{2\omega_0^2}\right) \tag{S7}$$

$$g(\theta) = \frac{1}{\sum_j N(\theta_j)} N(\theta_i)\,\delta(\theta-\theta_i) \tag{S8}$$

Their convolution $(f*g)(\theta)$ can be expressed as:

$$(f*g)(\theta) = \int_{-\infty}^{\infty} f(\theta')\, g(\theta-\theta')\,\mathrm{d}\theta' \tag{S9}$$

$$= \frac{1}{\sqrt{2\pi\omega_0^2}} \frac{1}{\sum_i N(\theta_i)} \int_{-\infty}^{\infty} \exp\left(-\frac{(\theta'-\theta_0)^2}{2\omega_0^2}\right) N(\theta_i)\,\delta(\theta-\theta'-\theta_i)\,\mathrm{d}\theta' \tag{S10}$$

$$= \frac{1}{\sqrt{2\pi\omega_0^2}} \frac{1}{\sum_i N(\theta_i)} N(\theta_i) \int_{-\infty}^{\infty} \exp\left(-\frac{(\theta'-\theta_0)^2}{2\omega_0^2}\right) \delta(\theta'-(\theta-\theta_i))\,\mathrm{d}\theta' \tag{S11}$$

$$= \frac{1}{\sqrt{2\pi\omega_0^2}} \frac{1}{\sum_i N(\theta_i)} N(\theta_i) \exp\left(-\frac{(\theta-(\theta_0+\theta_i))^2}{2\omega_0^2}\right) \tag{S12}$$

resulting again in a Gaussian distribution function with a shifted expectation value $\theta-(\theta_0+\theta_i)$ and a variable weighting factor $N(\theta_i)/\sum_i N(\theta_i)$.

S4 Convolution of two Gaussian functions

Let $f(\theta)$ and $g(\theta)$ be two normalized Gaussian functions with arbitrary central moments θ_0 and θ_1 and standard deviations ω_0 and ω_1.

$$f(\theta) = \frac{1}{\sqrt{2\pi\omega_0^2}} \exp\left(-\frac{(\theta-\theta_0)^2}{2\omega_0^2}\right) \tag{S13}$$

$$g(\theta) = \frac{1}{\sqrt{2\pi\omega_1^2}} \exp\left(-\frac{(\theta-\theta_1)^2}{2\omega_1^2}\right) \tag{S14}$$

Their convolution $(f*g)(\theta)$ can be expressed as:

$$
\begin{aligned}
(f*g)(\theta) &= \int_{-\infty}^{\infty} f\left(\theta'\right) g\left(\theta-\theta'\right) \mathrm{d}\theta' && \text{(S15)}\\
&= \frac{1}{2\pi\sqrt{\omega_0^2\omega_1^2}} \int_{-\infty}^{\infty} \exp\left(-\frac{\left(\theta'-\theta_0\right)^2}{2\omega_0^2}\right) \exp\left(-\frac{\left(\theta-\theta'-\theta_1\right)^2}{2\omega_1^2}\right) \mathrm{d}\theta' && \text{(S16)}\\
&= \frac{1}{2\pi\sqrt{\omega_0^2\omega_1^2}} \int_{-\infty}^{\infty} \exp\left(-\frac{\omega_1^2\left(\theta'-\theta_0\right)^2+\omega_0^2\left(\theta'-\left(\theta-\theta_1\right)\right)^2}{2\omega_0^2\omega_1^2}\right) \mathrm{d}\theta' && \text{(S17)}
\end{aligned}
$$

The exponent can be converted further:

$$
\begin{aligned}
E &= \frac{\omega_1^2\left(\theta'-\theta_0\right)^2+\omega_0^2\left(\theta'-\left(\theta-\theta_1\right)\right)^2}{2\omega_0^2\omega_1^2} && \text{(S18)}\\
&= \frac{\left(\omega_0^2+\omega_1^2\right)\theta'^2-2\left(\omega_1^2\theta_0+\omega_0^2\left(\theta-\theta_1\right)\right)\theta'+\omega_1^2\theta_0^2+\omega_0^2\left(\theta-\theta_1\right)^2}{2\omega_0^2\omega_1^2} && \text{(S19)}\\
&= \frac{\left[\left(\omega_0^2+\omega_1^2\right)\theta'-2\left(\omega_1^2\theta_0+\omega_0^2\left(\theta-\theta_1\right)\right)\theta'\right]}{2\omega_0^2\omega_1^2}+\frac{\omega_1^2\theta_0^2+\omega_0^2\left(\theta-\theta_1\right)^2}{2\omega_0^2\omega_1^2} && \text{(S20)}\\
&= \frac{\left[\sqrt{\omega_0^2+\omega_1^2}\,\theta'-\frac{\omega_1^2\theta_0+\omega_0^2(\theta-\theta_1)}{\sqrt{\omega_0^2+\omega_1^2}}\right]^2}{2\omega_0^2\omega_1^2}-\frac{\left[\frac{\omega_1^2\theta_0+\omega_0^2(\theta-\theta_1)}{\sqrt{\omega_0^2+\omega_1^2}}\right]^2}{2\omega_0^2\omega_1^2}+\frac{\omega_1^2\theta_0^2+\omega_0^2\left(\theta-\theta_1\right)^2}{2\omega_0^2\omega_1^2} && \text{(S21)}\\
&= \frac{\left(\omega_0^2+\omega_1^2\right)\left[\theta'-\frac{\omega_1^2\theta_0+\omega_0^2(\theta-\theta_1)}{\omega_0^2+\omega_1^2}\right]^2}{2\omega_0^2\omega_1^2}+\frac{\omega_1^2\theta_0^2+\omega_0^2\left(\theta-\theta_1\right)^2-\frac{\left[\omega_1^2\theta_0+\omega_0^2(\theta-\theta_1)\right]^2}{\omega_0^2+\omega_1^2}}{2\omega_0^2\omega_1^2} && \text{(S22)}\\
&= \frac{1}{2}\left(\frac{1}{\omega_0^2}+\frac{1}{\omega_1^2}\right)\left[\theta'-\frac{\omega_1^2\theta_0+\omega_0^2\left(\theta-\theta_1\right)}{\omega_0^2+\omega_1^2}\right]^2+\frac{1}{2}\frac{1}{\omega_0^2+\omega_1^2}\left[\theta_0+\left(\theta-\theta_1\right)\right]^2 && \text{(S23)}\\
&= \frac{1}{2}r^2\left[\theta'-s\right]^2+\frac{1}{2}t && \text{(S24)}
\end{aligned}
$$

When using

$$
\int_{-\infty}^{\infty}\exp\left(-\frac{r^2\left(\theta-s\right)^2}{2}\right)\mathrm{d}\theta=\frac{\sqrt{2\pi}}{r} \tag{S25}
$$

one gets

$$
(f*g)(\theta)=\frac{1}{\sqrt{2\pi\left(\omega_0^2+\omega_1^2\right)}}\exp\left(-\frac{\left(\theta-\left(\theta_0+\theta_1\right)\right)^2}{2\left(\omega_0^2+\omega_1^2\right)}\right), \tag{S26}
$$

which itself is a normalized Gaussian function with a shifted central moment ($\theta_c=\theta_0+\theta_1$) and a broadened width ($\omega^2=\omega_0^2+\omega_1^2$).

S5 Alternative sample preparations and DACs

As mentioned in the main article, alternative approaches appear to be noteworthy and are provided in the following. To achieve better support of the fibers, and hence obtain an improved geometrical alignment, different approaches can be imagined: Instead of employing one single gasket one can use two (thinner ones) for clamping the silk grating between them. Thus, the upper side of the upper gasket and the lower side of the lower counterpart will be deformed by the diamonds whereas the inner sides of the gaskets will maintain its shape supporting the grating (Figure 1a). Pre-indentation of a two-layered gasket does not cause any problems in our study, but drilling a bore on exactly the same position into both gaskets and placing them into the DAC emerges to be difficult. Remedy could be provided by employing guiding pins in both the drilling device as well as the DAC.[1]

Another approach could be provided by a vessel developed by Oger *et al.*.[2] Allowing for better microscopy imaging the pressure cell contains one diamond anvil and a flat diamond window instead of the second anvil. Similar to the former technique a grating of silk could be clamped between this window and the gasket experiencing better support through their flat surfaces (Figure 1b). Due to an unfavorable asymmetric construction hydrostatic pressure is limited up to 2 GPa.[2]

Thirdly, as employed initially for electrical measurements inside a DAC Gonzalez *et al.*[3], as well as, Patel *et al.*[4] used double-bevelled culets or culets with rounded edges, respectively, to prevent cutting wires which would also be helpful to prevent possible cutting of fibres. Unfortunately, such DACs or diamonds were not available for this study. So, validating these techniques remains for future prospects.

In contrast, casting a grating into an appropriate medium is also an alternative strategy. One advantage is that the casting medium supports the grating and retains its alignment. On the other hand, the casting medium should be rather solid, hence, hydrostatic conditions could be disturbed while, in addition, the support's material produces a background signal.

However, for the techniques introduced in the present work it is crucial to employ a low-viscous, good-wetting pressure transmitting medium. Highly viscous or even solid media can generate additional mechanical load on the sample while closing the pressure cell stressing the grating further. For this purpose paraffin oil is chosen, because it exhibits a higher viscosity than ethanol or mixtures of it and can be assumed as limit of viscosity. In principle, the presented methods should work fine with gaseous pressure transmitting media, as Argon, Helium, Neon, or Nitrogen for instance, or with liquid media, as ethanol-water or methanol-ethanol-water mixtures.[5] Glycerin or Vaseline, instead, seems to be too viscous. Additionally, these methods are not limited to infrared spectroscopy solely; Raman spectroscopy or X-ray and neutron scattering measurements are thinkable, too.

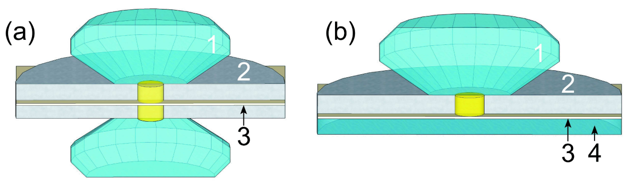

Fig. 1 Scheme of two alternative approaches for pressure-dependent measurements of the IR-dichroism in macromolecular fibers. (a) Two diamonds (1) are pressing against a double-layered gasket (2) supporting the grating (3). (b) One diamond anvil (1) is acting against a diamond window (4) while the grating (3) is supported by the window and the gasket (2).

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