

# Neutron Diffraction on Bicellar Mixtures Aligned by an *In-Situ* Temperature-Controllable Shear Flow Device

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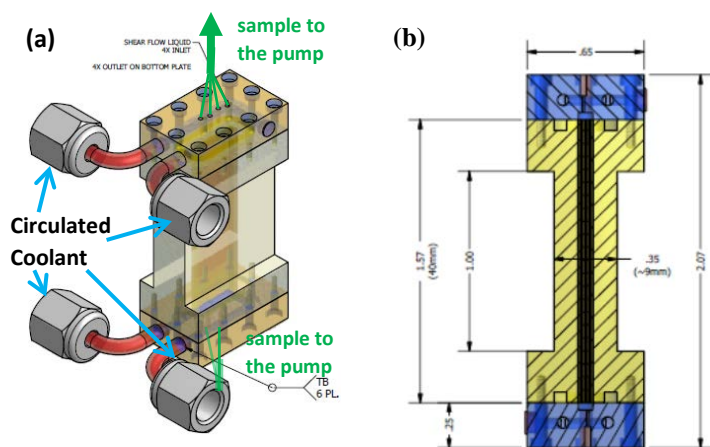
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In order to study the deformation and orientation of molecules or aggregates in solution due to the hydrodynamic interaction under flows, CNBC and UCONN researchers co-developed a *T*-controllable shear flow cell as shown in Fig 1. The interior of the cell is divided into three compartments by parallel Si wafers (each has a dimension of 40 mm x 0.3 mm x 10 mm). The total required sample amount is less than 1 mL. The temperature of the cell is determined by the top and bottom blocks which contain circulated coolant whose temperature is controlled by an external water bath. Both unidirectional and oscillational flows are achievable by the setting of the pump and the attainable shear rate is between 0.03 and 90,000 s<sup>-1</sup>.

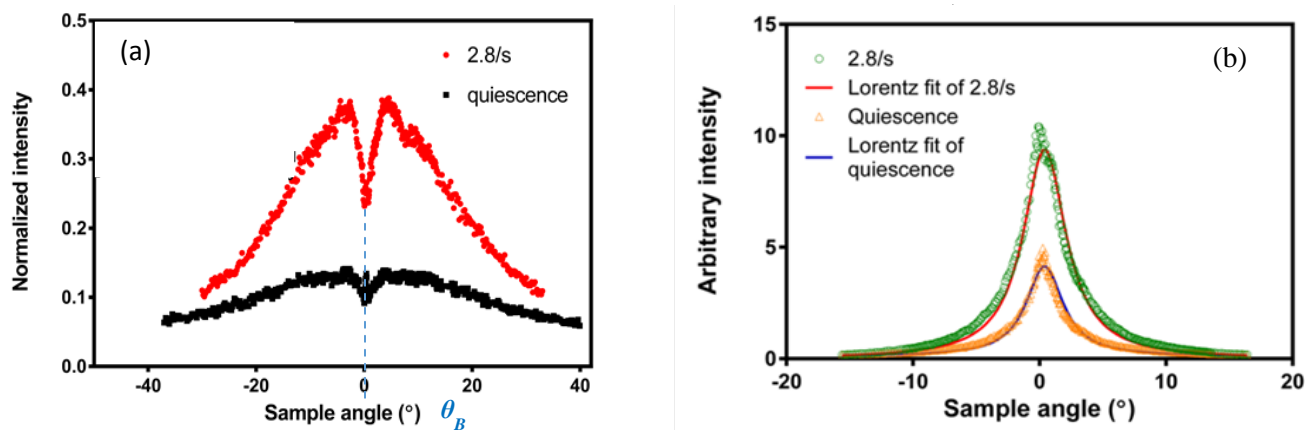


**Figure 1** (a) The shear flow cell with two *T*-controlling blocks (top and bottom) and (b) detailed side-view of the cell (blue shaded rectangles are *T*-controlling blocks).

The unit was validated by a lipid “bicellar” mixture which contains a long-chain dimyristoyl phosphatidylcholine (DMPC), a long-chain dimyristoyl phosphatidylglycerol (DMPG) and a short-chain dihexanoyl phosphatidylcholine (DHPC) with a DMPC:DMPG:DHPC molar ratio of 2.88 : 0.32 : 1.0. The final lipid concentration of the solution is 20 wt.%. A structural transition from non-alignable nanodiscs to alignable perforated membranes upon increasing temperature will take place at above 23 °C [1]. The neutron diffraction result of the sample at 31 °C reveals three Bragg peaks, indicative of a lamellar structure (the Bragg angle,  $\theta_B$  is 0.4° and the first order Bragg peak is at 0.037 Å<sup>-1</sup>).

To evaluate the degree of alignment of the sample under the shear flow, the “rocking curve” measurement was taken by setting the detector angle at the Bragg’s peak position (i.e.,  $2\theta_B$ ). Fig 2(a) shows the raw data of the rocking curves of sample at 31 °C under a shear flow of  $\dot{\gamma} = 2.8$  s<sup>-1</sup> and quiescent state after the flow, respectively. Both cases share a common pattern: a peak located exactly at the Bragg angle,  $\theta_B$  and a valley followed by a broad shoulder are found as  $\theta$  extends outward on each side of  $\theta_B$ . The two valleys are attributed to the strong absorption of the incident and diffracted neutrons at  $\theta = 0$  and  $2\theta_B$ , respectively. Similar patterns with less absorption were also observed in the cases of highly aligned lipid bilayers using a flat substrate [2]. The shoulders represent the azimuthally misaligned population of bilayers. In the case of substrate-aligning membranes, there is only a small portion of the bilayers deviating from the Bragg’s condition within a reasonably narrow distribution

(FWHM  $< 0.2^\circ$ ) [3], while due to the higher mobility in the solution a much larger population of bilayers are misaligned with a broader distribution, resulting in much broader shoulders.



**Figure 2** (a) The raw rocking curves of the DMPC/DHPC/DMPG mixture at  $T = 31^\circ\text{C}$  at  $\dot{\gamma} = 2.8\text{ s}^{-1}$  (circles), and after the flow ceased (squares). (b) The corrected rocking curves of the raw data in Fig 5 at  $\dot{\gamma} = 2.8\text{ s}^{-1}$  (green), and after the flow ceased (orange). The Lorentz fits of corrected data at  $\dot{\gamma} = 2.8\text{ s}^{-1}$  (red), and after the flow ceased (blue).

Three factors have been taken into account in order to correct the raw rocking curves: the absorption of the sample in a rectangular geometry, the portion of the neutrons that illuminate the sample and the scattering volume “viewed” by the detector. Detailed derivation can be found in ref [4]. The corrected rocking curves are shown in Fig 2(b) and best fitted by Lorentz equation, resulting in a full width of half maxima (FWHM) of  $4.1^\circ$  and  $3.5^\circ$  under shear flow and at quiescence, respectively. This result seems to suggest a slightly better alignment in the case of quiescent state, possibly attributed to fewer disturbances of the aligned bilayers near the surfaces. However, Fig 2(b) also indicates a significantly larger population of the bilayers induced by the shear flow aligned over the range of  $\pm 10^\circ$  compared to that at quiescence. Since the sample compositions and amounts were the same under the shear and at the quiescent conditions, the integrated area under the rocking curves over from  $-180^\circ$  to  $180^\circ$  should be identical. Thus, the background of the sample at quiescence is expected to be higher than that under the shear flow at higher sample angles.

The interpretation slightly differs from the previous report based on SANS data, which revealed up to the forth-order Bragg’s peak in a quiescent sample after oscillating shear – being interpreted as highly-aligned membranes [5,6]. The “rocking curves” obtained in the current shear flow device provide further insight to the degree of the alignment throughout the sample. *It should be noted that the existence of higher-order Bragg’s peaks does not necessarily indicate highly-aligned sample.* In fact, randomly-oriented samples may also result in higher order Bragg’s peaks as there is always a portion of the bilayers oriented in a way satisfied with the Bragg conditions.

We have shown that the newly-designed  $T$ -controlled shear flow device is suitable for *in-situ* ND measurements. The shear strain rate can be easily adjusted through controlling the flow rate produced by the external pump. The device allows the studies on the hydrodynamic effect on the nano-structures of materials and the conformation of molecules associated with aligned membranes.

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