Electronic Supplementary Material (ESI) for Chemical Communications


# SUPPORTING INFORMATION

## Flow-Alignment of Bicellar Lipid Mixtures: Orientations of Probe Molecules and Membrane-Associated Biomacromolecules in Lipid Membranes Studied with Polarized Light

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## Materials and methods

**Materials.** 1,2-dicaproyl-sn-glycero-3-phosphocholine (DHPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) were purchased as chloroform solutions from Avanti Polar Lipids (Alabaster, AL, USA). Pyrene (Py), sodium dodecyl sulphate (SDS), Retinoic acid (RetA) and cytochrome c (cyt *c*) were purchased from Sigma.

**Sample preparation.** Compositions of phospholipid samples are characterized by the following descriptors: total lipid concentration, $c_L$, lipid molar ratio, $q=[DMPC]/[DHPC]$, and total weight percent of the lipids, $l$. Phospholipid samples were prepared as described elsewhere.[1] Prior to LD measurements the following cycle of sample equilibration was performed three times for all lipid mixtures: heating up to ~40°C, vortexing, cooling to ~5°C and vortexing. Stock solutions of Py and RetA in ethanol were added to the lipid sample to obtain the required molar ratio. Final volume of ethanol in the samples was less than 2% (v/v). Stock solution of cyt *c* in water was added to the lipid sample to achieve required molar ratio. Upon addition of each membrane probe, samples were vortexed and left for equilibration for at least 1 hour prior to the measurements.
Following sample compositions and probes were used in this study:

**(a)** 1. [Py]/ [Total lipids]=1:1000 in DMPC/DHPC ($l$=3%, $q$=3.2)
2. [RetA]/ [Total lipids]=1:1000 in DMPC/DHPC ($l$=3%, $q$=3.2)
**(b)** [Py]/ [Total lipids]=1:10 000 in DMPC/DHPC ($l$=20%, $q$=3.2)
**(c)** 1. [Py]/[Total lipids]= 1:8000 in DMPC/DHPC/SDS ($l$=3%, $q$=3.2, [DMPC]/[SDS]=10:1)
2. [Cyt *c*]/[Total lipids]= 1:1000 in DMPC/DHPC/SDS ($l$=3%, $q$=3.2, [DMPC]/[SDS]=10:1)

**Flow Linear Dichroism (LD) Spectroscopy.** LD spectrum for sample **(b)** was recorded on Jasco-720 spectropolarimeter. LD spectra from samples **(a)** and **(c)** were recorded on a Chirascan CD spectrometer equipped with an LD detector. A custom made outer cylinder rotation Couette flow cell (volume – 2ml, pathlength 1mm) connected with thermostated water bath was used for sample **(c)**. Samples **(a)** and **(b)** were oriented in a 100 µL custom made outer rotation Couette flow cell with pathlength of 0.54 mm. All recorded data were normalized to 1mm pathlength.
A rotation control device was used to apply shear gradients in the range of 12-3100 $s^{-1}$. Spectra were collected between 180-600nm for cyt *c* and RetA and between 200-400nm for Py in 1 nm increments and a scan speed of 100nm/min. At least three

spectra from each measurement were accumulated for an average. Baselines at zero shear gradients were recorded and subtracted from all spectra. Due to the turbidity of sample **(a1)** an additional subtraction of a turbidity contribution was made by fitting an exponential function of second order (Figure S9).

**Absorption Spectroscopy.** Absorption spectra, with 100 nm/min scanning speed and 1 nm data steps, were recorded at room temperature using a Cary 50 Bio UV-Vis spectrophotometer (Varian) and a quartz cell with 1mm pathlength.

**Dynamic Light Scattering (DLS).** DLS measurements were performed on a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK). The time-dependent autocorrelation function for each measurement was averaged from 10 to 15 measurements, with 10 s measurement times. The samples were illuminated at 633 nm by He/Ne laser and the detection of the scattered light was made at the angle of 173°. Measurements were performed for the temperature interval of 15°C-45°C with 2°C increments and 30 minutes of equilibration time. The analysis of the autocorrelation functions was done using the built-in software from Malvern Instruments. A refractive index of 1.424 was used for the samples.

**Calculation of orientation parameter.** The macroscopic orientation parameter, S, was calculated as previously described.[2, 3] LD and absorbance data at 23 °C and $900s^{-1}$ for sample **(a2)** containing RetA (1:1000) and DMPC/DHPC ($q$=3.2; $l$=3%) was used due to the highest LD signal strength (Figure S2). Orientation parameter for the described conditions was calculated to S=0.3.

## Figures

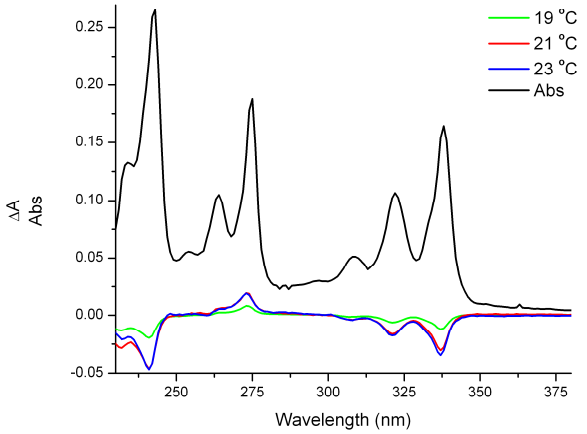


**Figure S1**. Absorbance spectrum of Py, sample **(a1),** and LD spectra between 19°C and 23°C at a shear gradient of 900 $s^{-1}$

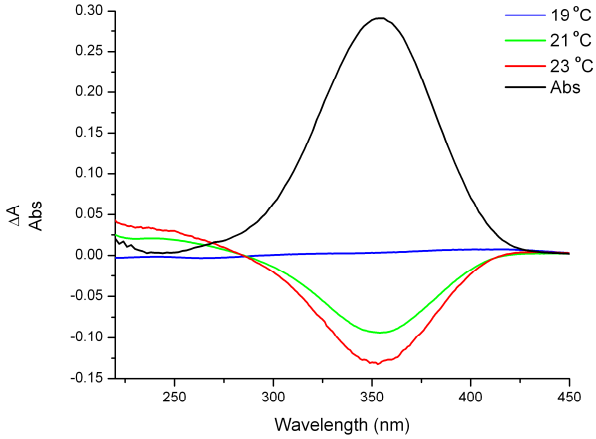


**Figure S2**. Absorbance spectrum of RetA, sample **(a2)**, and LD spectra between 19°C and 23°C at a shear gradient of 900 $s^{-1}$

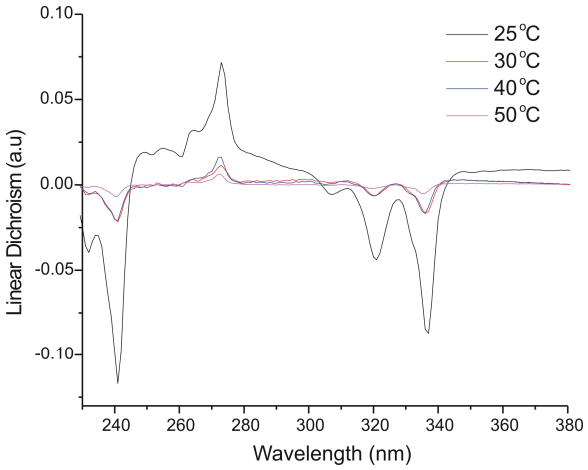


**Figure S3** LD spectra of sample (**a1**) as the function of temperature in the range of 25-50°C and using an applied shear gradient of $900s^{-1}$. Pyrene to lipid ratio is 1:500.

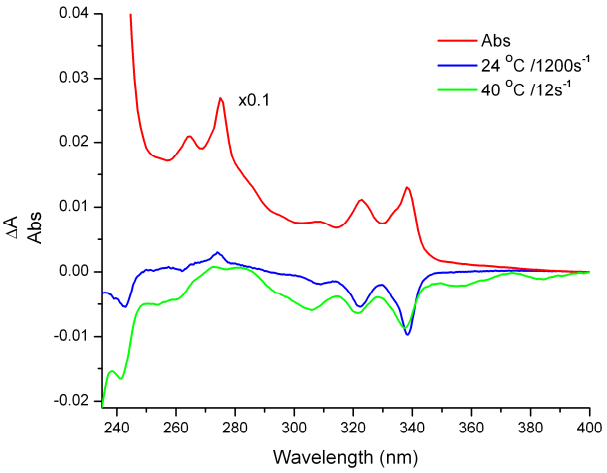


**Figure S4**. Absorbance spectrum and LD of sample **(b)** at 24°C (shear gradient $1200s^{-1}$) and 40°C (shear gradient $12s^{-1}$)

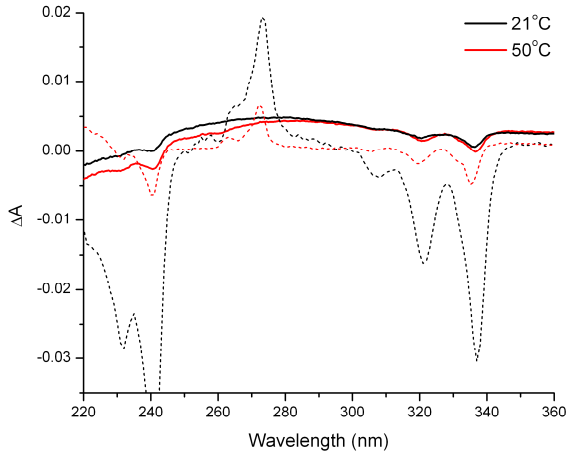


**Figure S5.** LD spectra of the DHPC/DMPC sample (**a1**) (dashed lines) and a sample with DMPC only (solid lines), both having 3wt% lipid content. Pyrene to lipid ration is 1:1000, applied shear gradient is $900s^{-1}$.

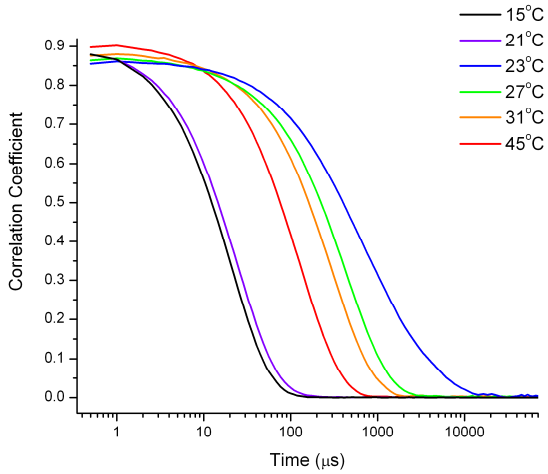


**Figure S6** Correlation coefficients from the autocorrelation functions measured using DLS.

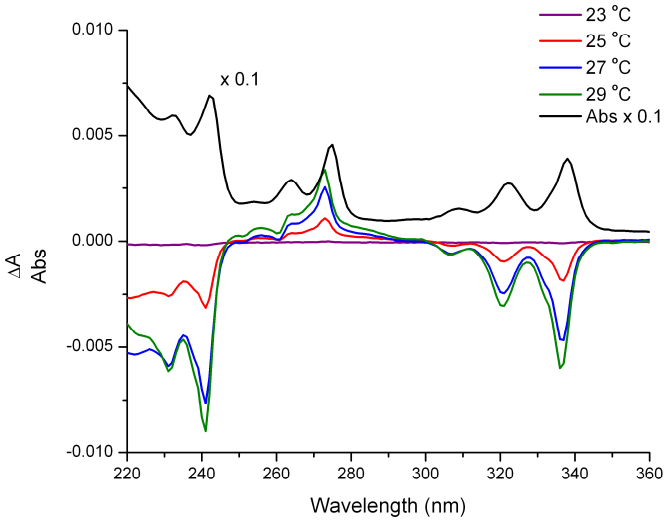


**Figure S7**. Absorbance spectrum and LD spectra of Py, sample **(c1)**, between 23°C and 29°C at a shear gradient of ~600 $s^{-1}$

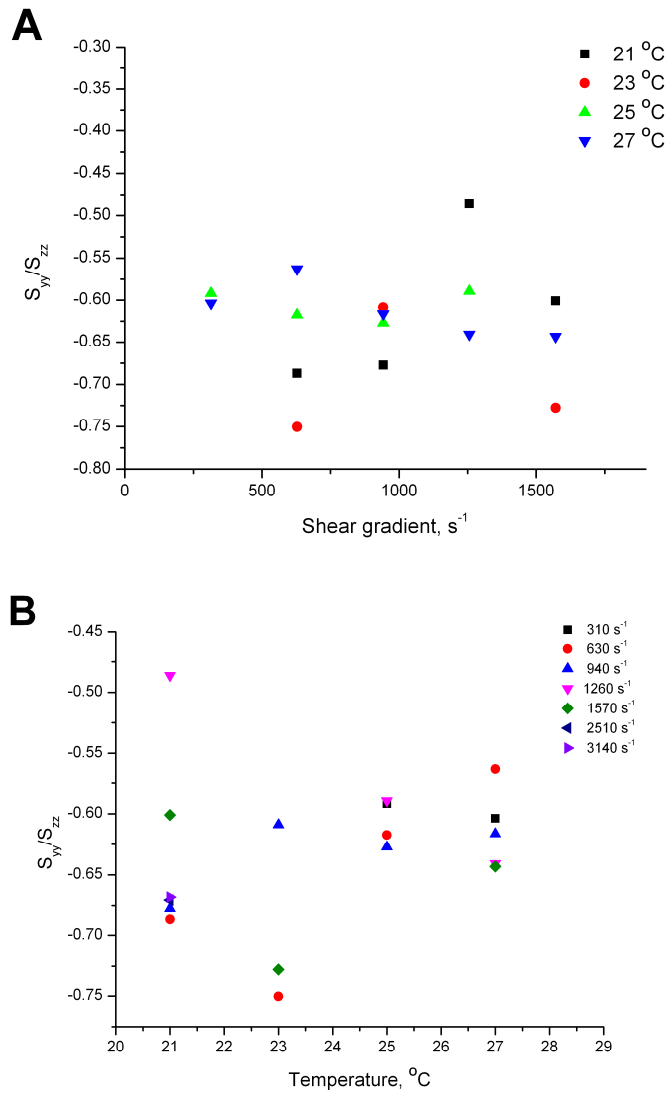


**Figure S8**: Ratios between orientation factors of pyrene's transition moments $S_{yy}$ (272nm) and $S_{zz}$ (339nm) plotted as function of shear gradient (**A**) and as function of temperature (**B**) for sample **(a1)**

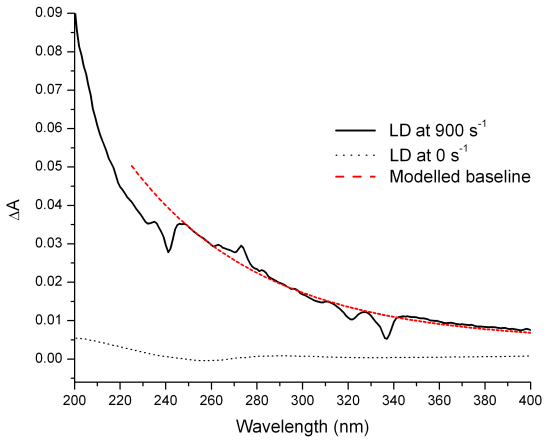


**Figure S9** Correction of the turbidity contribution for sample (**a1**); LD spectra were recorded at a shear gradient of 900 $s^{-1}$ (solid line) and 0 $s^{-1}$ (dotted line). The turbidity contribution line is modelled by second order exponential function (red dashed line).

## References in the Supporting Information

1. P. Beck, M. Liebi, J. Kohlbrecher, T. Ishikawa, H. Ruegger, P. Fischer, P. Walde and E. Windhab, *Langmuir*, 2010, **26**, 5382-5387.
2. E. K. Esbjorner, K. Oglecka, P. Lincoln, A. Graslund and B. Norden, *Biochemistry*, 2007, **46**, 13490-13504.
3. F. R. Svensson, P. Lincoln, B. Norden and E. K. Esbjorner, *J. Phys. Chem. B*, 2007, **111**, 10839-10848.