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Letter

Interactions of a Photochromic Spiropyran with Liposome Model Membranes

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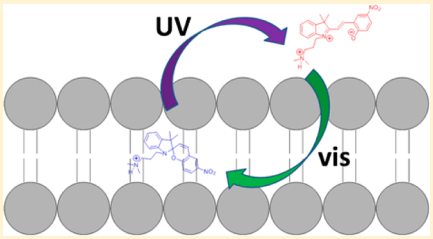
Supporting Information

ABSTRACT: The interactions between anionic or zwitterionic liposomes and a water-soluble, DNA-binding photochromic spiropyran are studied using UV/vis absorption and linear dichroism (LD) spectroscopy. The spectral characteristics as well as the kinetics of the thermal isomerization process in the absence and presence of the two different liposome types provide information about the environment and whether or not the spiropyran resides in the liposome membrane. By measuring LD on liposomes deformed and aligned by shear flow, further insight is obtained about interaction and binding geometry of the spiropyran at the lipid membranes. We show that the membrane interactions differ between the two types of liposomes used as well as the isomeric forms of the spiropyran photoswitch.



INTRODUCTION

DNA-binding molecules are generally considered potential candidates for anticancer treatment in medicinal research,[1−3] though their delivery efficiently and specifically to the nucleic acid target, including crossing the cellular membrane, remains a major challenge.[4] Molecules for which the DNA binding may be tuned or triggered by internal or external stimuli are therefore of great interest, as they could potentially lead to novel strategies for delivery and better targeted treatments.[5−11] One such compound is the photochromic spiropyran **1** previously studied in this laboratory (Figure 1).[12−14]

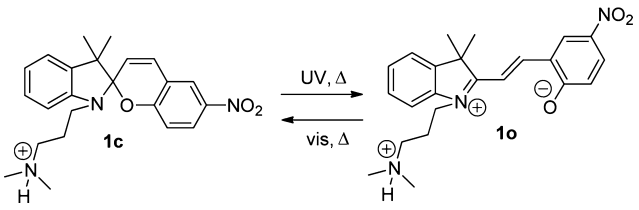


Figure 1. Structures and isomerization scheme of spiropyran **1**. The closed spiro form **1c** and the open merocyanine form **1o** are shown.

A photochromic compound has two, more or less, thermally stable forms whose conversion into each other is light-controlled. In the case of the spiropyran class of compounds, these forms are the biplanar spiro form **1c**, referred to as the closed form, and the planar open merocyanine form **1o**. Using UV-light, **1c** can be isomerized to **1o**, and the reverse process can be triggered by irradiating the sample with visible light. At room temperature in the dark, a thermal equilibrium is established with a time constant of about 10 h yielding a ca. 50/50 composition **1c**/**1o**. Previous studies performed in this laboratory have shown that spiropyran derivatives similar to **1** bind to DNA only after protonation of the open merocyanine form **1o**, whereas the closed spiro form **1c** shows no signs of binding.[12,13] Since most cancer cells are known to be somewhat more acidic compared to the healthy cells, UV light and pH dependent triggering mechanisms could potentially provide the basis for strategies for targeting and attenuating tumors. Photocontrolled binding to nucleic acids as well as binding to and translocation through cell membranes are, therefore, particularly interesting properties in this context.

The lipid bilayers of large unilamellar lipid vesicles (LUVs), also known as liposomes, are often used as model systems for cellular membranes.[15] Despite considerable differences between the homogeneous lipid bilayer of a liposome and the very complex plasma membrane of a cell, the interactions of potential probe molecules with liposomes can provide vital information about the probe concerning a range of variables, such as binding affinity, local orientation, and spectral variations when the probe is entering into or exits the membranes.[16,17] We here show that linear dichroism spectroscopy may provide unique insight into how the spiropyran, in both its closed and open forms, interacts with neutral as well as negatively charged lipid vesicle membranes.

Linear dichroism (LD) is the difference in absorption of linearly polarized light parallel and perpendicular to a macroscopic reference axis defined by hydrodynamic flow or other aligning force fields.[16]

$$\mathrm{LD} = A_{\parallel} - A_{\perp} \tag{1}$$

LD spectroscopy is a useful technique for studying, for example, the binding modes of probe molecules relative to biopolymers, lipid lamellar membranes, or elongated micelles.[17−19] Also solutes in the lipid bilayer of liposomes have been possible to study.[20,21] Since LD spectroscopy requires an anisotropic

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sample, a shear flow is used to elongate and align the liposomes. The degree of alignment is normally very small but sufficient for an anisotropic absorption measurement to yield results on the probe absorption and the orientation of chromophoric solutes at or inside the membrane. Refractive index matching with sucrose is used to mask the scattering of the liposomes as well as enhancing the orientation and LD signal due to increased solution viscosity.[22]

In this study, the interactions of spiropyran **1** with liposomes made from anionic or zwitterionic lipids are investigated using UV/vis-absorption and LD. The results show that while **1c** binds to both anionic and zwitterionic liposomes, **1o** only binds to liposomes made from anionic lipids. We can also conclude that **1o** is released from the zwitterionic liposomes upon UV-induced isomerization from **1c**. From the orientational behavior and the isomerization kinetics of the spiropyrans in the presence of the two different membranes, important insight is obtained into how the two forms of this molecule are interacting with various membrane contexts. This is information useful both from the nanotechnical point of view, for example, for the construction of photoactivatable supramolecular structures, and from a biomedical angle, for example, for steered DNA-binding or membrane-translocation of cancer targeting drug molecules.

MATERIALS AND METHODS

Spiropyran **1** used in this study was synthesized using the procedure detailed in a previous article.[14] Lipids were purchased from Avanti Polar Lipids and used without further purification. Sucrose and buffer salts were from Sigma and used without further purification. The absorption measurements were carried out on a Varian Cary 50 Bio instrument fitted with a multicell holder, a temperature control, and a magnetic stirrer. The light source used for the UV-induced opening process (**1c** → **1o**) was a hand-held UV-lamp (UVGL-25, 254 nm, 700 μW/cm^2). The linear dichroism measurements were carried out on an Applied Photophysics Chirascan spectrophotometer equipped with a linear dichroism detector, using a Couette cell. Sucrose (50 wt %) was added to the samples to match the solution and liposome refractive indices and to increase the viscosity of the solution.

Liposomes were prepared using a previously described procedure.[16] A volume of 500 μL of lipid (25 mg/mL in $CHCl_3$) was diluted with another 500 μL of $CHCl_3$ and the solvent evaporated under reduced pressure. The lipid films were put under high vacuum overnight and then dissolved in a sodium phosphate buffer (100 mM, pH 7.48). The sample was subjected to a freeze−thaw cycle using liquid nitrogen to rapidly freeze and a heating block (37 °C) to thaw the sample a total of five times each. The solution was then extruded through a filter with 100 nm (diameter) pores 21 times.

LD samples were prepared by dissolving 1.5 g of sucrose in 1.35 mL of sodium phosphate buffer. Then 100 μL of liposome solution was added, and finally 50 μL of spiropyran (0.6 mg in 1 mL of Milli-Q water) was added. UV/vis samples were prepared as above, omitting only the sucrose.

To determine the direction of the electric dipole transition moments responsible for light absorption of the closed and open conformations of the spiropyran, molecular structures of **1c** and **1o** were optimized at the B3LYP/6-31G(d) level, followed by a time-dependent density functional theory (TDDFT) calculation at the B3LYP/6-31+G(d,p) level of theory, using the Gaussian 03 software package.[23] For both conformers, these show that the electronic transition, responsible for the first absorption band, is polarized near parallel with the molecular long-axis. For more details see the Supporting Information.

RESULTS

The results from UV/vis absorption studies of the solvatochromic shifts of the absorption maxima and the thermal isomerization kinetics of **1o** → **1c** in the presence of zwitterionic and anionic liposomes are presented in Table 1 and Figures 2 and 3.[24] When going from pure buffer solvent to the zwitterionic POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), the wavelength of the **1o** absorption maximum is only slightly affected (red-shift from 510 to 514 nm) and the isomerization process **1o** → **1c** is not associated with any significant changes. By contrast, for the anionic POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol), sodium salt) the **1o** absorption maximum is substantially red-shifted, from 510 to 529 nm, and the corresponding isomerization kinetics is dramatically accelerated.

Table 1. Wavelength of the absorption Maximum of 1o in Different Environments

	Milli-Q water	buffer	POPC	POPG
$\lambda_{max,1o}$ (nm)	510	510	514	529, 546[a]
corresponding E_T^N	1	1	0.99	0.84, 0.66[a]

[a]Determined from wavelength position of LD maximum in comparison to absorption maximum.

To further investigate the membrane binding, linear dichroism measurements were carried out (Figure 2b,c). The LD results in the zwitterionic liposome POPC show no traces of any bound **1o**, zero LD between 450 and 600 nm, but a strong positive LD for **1c** at wavelengths shorter than 400 nm. In contrast, the results for the anionic liposome POPG show LD peaks for **1o** as well as **1c** and the LD maximum for **1o** is around 546 nm.

The reduced linear dichroism, which is the linear dichroism divided by the corresponding isotropic absorption, can provide more detailed information about the chromophore orientation. For liposome bound probes, the LD^r can be expressed as[16]

$$LD^r = 3S\frac{1 - 3cos^2\,\alpha}{4} \quad (2)$$

Here, S is a macroscopic orientation factor for the membrane normal with respect to the "parallel" direction of the laboratory system as defined by eq 1, and α represents the angle that the transition dipole moment, responsible for the light absorption, makes with the membrane normal. The apparent value α when solving eq 2 corresponds to an average of $\langle cos^2\,\alpha\rangle$ over the angular distribution that dynamics and possible structural heterogeneity may give rise to. To calibrate the orientation factor of the shear-aligned liposome membrane, retinoic acid was used as a probe, assumed to adopt a perfect orientation parallel to the membrane normal ($\alpha = 0°$). The observed LD^r = −0.005 for retinoic acid corresponds to $S = 0.0033$; thus, for **1c**, whose LD^r = +0.0016 in zwitterionic POPC, the angle toward the membrane normal is about 70°. The corresponding value for **1c** in anionic POPG is determined to 61°. In POPG, the LD^r of **1o** was 0.0011 at 546 nm, which (using the same value for S) corresponds to an angle toward the membrane normal of about 65°. From the correlation that the electric dipole transition moments are polarized very near parallel with the chromophore long-axis, these orientation angles may be considered to represent the respective alignments of the long-axis

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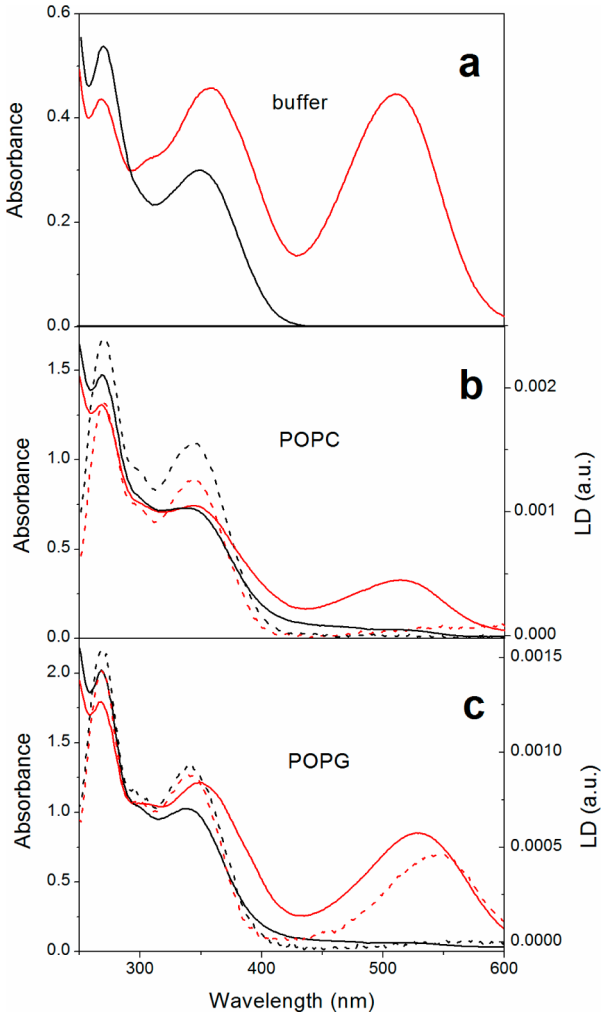

Figure 2. (a) Absorption spectra of **1** in buffer solution at pH 7.4 before (black solid line) and after (red solid line) UV irradiation at 254 nm. The photostationary distribution reached is ca. 40/60 **1c**/**1o**. Shown are also the corresponding absorption spectra of **1** in the presence of zwitterionic POPC (b) and anionic POPG (c) liposomes. The LD spectra before (black dashed line) and after (red dashed line) UV irradiation are also shown. The absence of any LD signal of **1o** in POPC suggests that there are no marked interactions of the open form with zwitterionic liposomes. By contrast with anionic POPG liposomes, **1o** displays strong (positive) LD in its absorption bands. The closed form **1c** shows LD and obviously interacts with both types of liposomes.

To estimate the depth of insertion of the molecule into the membrane, the wavelength of the absorption maximum of the solvatochromic open form **1o** in solvents of different polarities was studied.[26] The results are collected in Table 1. The E_T^N values of the different solvents were obtained from Reichardt[27] (see the Supporting Information for solvatochromic shifts for **1o** in various solvents). The results suggest that **1o** experiences a much less polar environment than in phosphate buffer when associated to anionic liposomes, whereas the polarity of the environment appears to be similar compared to the buffer solution when in the presence of zwitterionic liposomes.

DISCUSSION

For the anionic POPG liposomes, the spectroscopic and kinetic results paint a rather clear picture of their distinct interactions with spiropyran, for both the open and closed forms, and

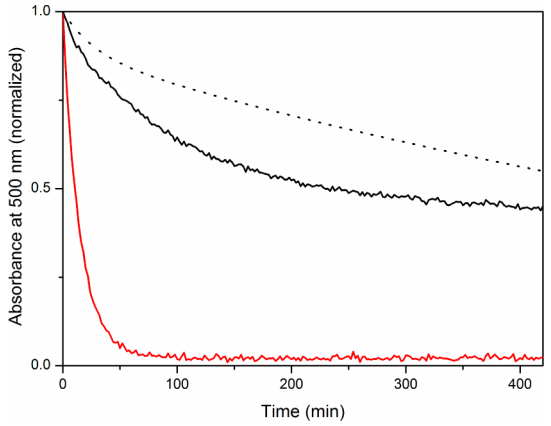

Figure 3. Absorption decays showing thermal isomerization of **1o** → **1c** in buffer at pH 7.4 (black dotted line), with POPC liposomes (black solid line) and with POPG liposomes (red solid line) present.[25]

regarding their preferred binding geometries and the catalytic effects of the lipid bilayers on isomerization. For the zwitterionic liposomes, on the other hand, only the closed form of spiropyran appears to be firmly associated to the membrane whereas the open form is by and large unbound.

The thermal isomerization **1o** → **1c** is greatly accelerated in the presence of the anionic POPG liposomes as compared to a buffered solution, and both **1c** and **1o** exhibit strong, positive peaks in the LD spectrum at the absorption bands for the first electronic transition, consistent with orientations of the long-axis of the chromophore preferentially parallel with the membrane. Furthermore, the wavelength of **1o** LD maximum at 546 nm immediately reveals the position of the absorption of the membrane-bound chromophores (since molecules free in solution will not contribute any LD). This wavelength in turn suggests that the E_T^N is about 0.66, representing, as expected, a much less polar environment than in the buffer. This could indicate that the main body of the electronic chromophore resides inside the lipid alkyl chain region, with only the polar edge pointing outward. Such an interaction would be in accord with experience of orientation behavior of planar chromophores in lipid membranes.[16] From a quantitative analysis of LD we can conclude that **1** binds to the membrane, and sits within the membrane with an angle of about 60−70° relative to the membrane normal, as sketched in Figure 4.

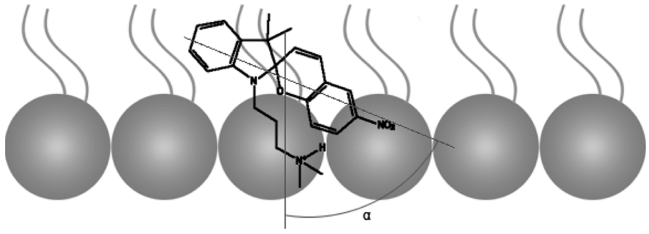

Figure 4. Schematic suggested orientation of the closed spiro form **1c** in a lipid membrane, based on α = 60−70° from LD data.

The near linear correlation observed between the wavelength of the absorption maximum of **1o** and the solvent polarity shows that the solvatochromic effect follows a behavior expected for electrostatic interactions possibly in part involving hydrogen bonding (see the Supporting Information).[28] Furthermore, the accelerated isomerization **1o** to **1c** is also in

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favor of the notion of a more hydrophobic environment, as it is established that the thermal decolorization process is faster in nonpolar environments.[29]

For the zwitterionic liposome POPC, the thermal isomerization process **1o** → **1c** is similar to what is observed when free in buffer (Figure 3). Here, too, the linear dichroism of **1c** indicates an angle toward the membrane normal of about 70°, but in this case the open form **1o** does not show any LD. There are three possible explanations for the lack of an LD signal: (i) **1o** does not bind to zwitterionic liposomes, (ii) there is no net orientation of **1o** when bound to the liposomes ("random orientation"), or (iii) the angle of the **1o** transition dipole toward the membrane normal is exactly the magic angle, 54.7°. Since the wavelength of the absorption maximum of **1o** is not shifted to any larger extent when zwitterionic liposomes are present, and the isomerization kinetics are not at all accelerated as much as when **1** binds to anionic liposomes, we conclude that (i) is the most likely explanation: The absence of LD is due to that there is no interaction between **1o** and the lipid membrane. This behavior means that **1c** binds to zwitterionic liposomes, but as soon it is isomerized into **1o** it is released into the bulk solution.

The behavior of **1** in zwitterionic and anionic lipid contexts is interesting from a number of angles. First, the way chromophoric solutes are solubilized in biological membranes is of crucial importance for understanding function of both photosynthetic systems as well as vision pigments where solvatochromic effects as result of subtle displacement effects relative to the surrounding dielectric may determine the photophysical properties. To disentangle these effects from electrochromic effects, that is, the perturbation of the internal electronic properties of a chromophore due to an external electric field, in a membrane is an important but challenging project. Second, and in particular for the photocontrollable spiropyrans, various opto-technical applications may exploit and piggyback on such effects in order to perform various functions in nanotechnical context. Third, in biotechnical and medicinal applications, the possibility to control membrane binding and possibly thereby also cellular uptake (another project we wish to vigorously pursue) offers interesting strategies for targeting specifically certain cells or cellular functions.

As to orientation of the spiropyran in/at the membrane, the aromatic ring system would, in case no polar or charged groups were present, be expected to orient itself parallel with the alkyl chains due to edgewise insertion in the lipid membrane, the normal orientation for an elongated perfectly nonpolar molecule. The observed, more orthogonal orientation, with the long-axis preferentially more parallel with than perpendicular to the membrane, is consistent with the heteroaromatic nature of the spiropyran chromophore. The closed nonplanar **1c** and the open rather planar **1o** structures of the chromophores can be anticipated to be oriented due to steric interactions (with the well-ordered alkyl chains of the membrane) and electrostatic interactions. The latter will tend to position the more polar edge of the open form toward the polar head region and the more nonpolar edge pointing inward the membrane. LD could be a way to verify this edge-wise orientation, had we had any transversal transition moments that could confirm that this dimension is preferentially aligned parallel with the membrane normal.

In conclusion, we have shown that spiropyran **1** displays dramatically different interactions with zwitterionic and anionic liposomes. Whereas the closed spiro form **1c** interacts with the membrane of both zwitter- and anionic liposomes, the open merocyanine form **1o** only interacts with anionic liposomes. The difference between zwitterionic and anionic lipid membrane system may be sought in the surface charge distribution. While the zwitterion dipole field is of a more local nature, the long-range effects of the electric fields of ligand charge are only partially shielded by counterions. The markedly different LD spectra for **1o** when in presence of zwitterionic (no LD) and anionic (strong positive LD) is interesting and has not been observed before.

■ ASSOCIATED CONTENT

Supporting Information

Computational details and solvatochromic shifts for **1o** in various solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Paul, A.; Bhattacharya, S. Chemistry and biology of DNA-binding small molecules. *Curr. Sci.* **2012**, *102*, 212−231.

(2) Dervan, P. B. Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235.

(3) Nelson, S. M.; Ferguson, L. R.; Denny, W. A. Non- covalent ligand/DNA interactions: minor groove binding agents. *Mutat. Res.* **2007**, *623*, 24−40.

(4) Dass, C. R.; Choong, P. F. M. Targeting of small molecule anticancer drugs to the tumour and its vasculature using cationic liposomes: lessons from gene therapy. *Cancer Cell Int.* **2006**, *6*.

(5) Berdnikova, D.; Fedorova, O.; Gulakova, E.; Ihmels, H. Photoinduced in situ generation of a DNA-binding benzothiazoloquinolinium derivative. *Chem. Commun.* **2012**, *48*, 4603−4605.

(6) Di Pietro, M. L.; Puntoriero, F.; Tuyeras, F.; Ochsenbein, P.; Laine, P. P.; Campagna, S. Photochemically driven intercalation of small molecules into DNA by in situ irradiation. *Chem. Commun.* **2010**, *46*, 5169−5171.

(7) Brieke, C.; Rohrbach, F.; Gottschalk, A.; Mayer, G.; Heckel, A. Light-Controlled Tools. *Angew. Chem., Int. Ed.* **2012**, *51*, 8446−8476.

(8) Mammana, A.; Carroll, G. T.; Areephong, J.; Feringa, B. L. A; Chiroptical Photoswitchable, D. N. A. Complex. *J. Phys. Chem. B* **2011**, *115*, 11581−11587.

(9) Mayer, G.; Heckel, A. Biologically active molecules with a "light switch". *Angew. Chem., Int. Ed.* **2006**, *45*, 4900−4921.

(10) Paramonov, S. V.; Lokshin, V.; Ihmels, H.; Fedorova, O. A. Influence of DNA-binding on the photochromic equilibrium of a chromene derivative. *Photochem. Photobiol. Sci.* **2011**, *10*, 1279−1282.

(11) Willner, I. Photoswitchable biomaterials: En route to optobioelectronic systems. *Acc. Chem. Res.* **1997**, *30*, 347−356.

(12) Andersson, J.; Li, S. M.; Lincoln, P.; Andréasson, J. Photoswitched DNA-binding of a photochromic spiropyran. *J. Am. Chem. Soc.* **2008**, *130*, 11836−11837.

(13) Hammarson, M.; Andersson, J.; Li, S. M.; Lincoln, P.; Andréasson, J. Molecular AND-logic for dually controlled activation of a DNA-binding spiropyran. *Chem. Commun.* **2010**, *46*, 7130−7132.
(14) Nilsson, J. R.; Li, S. M.; Önfelt, B.; Andréasson, J. Light-induced cytotoxicity of a photochromic spiropyran. *Chem. Commun.* **2011**, *47*, 11020−11022.
(15) Lasic, D. D.; Lipowsky, R.; ; Sackmann,E. Applications of liposomes. In *Handbook of Biological Physics*; North-Holland: Amsterdam, 1995; p 491−519.
(16) Nordén, B.; Rodger, A.; Dafforn, T. *Linear Dichroism and Circular Dichroism. A Textbook on Polarized-Light Spectroscopy*; The Royal Society of Chemistry: Cambridge, 2010.
(17) Hicks, M. R.; Kowalski, J.; Rodger, A. LD spectroscopy of natural and synthetic biomaterials. *Chem. Soc. Rev.* **2010**, *39*, 3380−3393.
(18) Adachi, R.; Yamaguchi, K.; Yagi, H.; Sakurai, K.; Naiki, H.; Goto, Y. Flow-induced alignment of amyloid protofilaments revealed by linear dichroism. *J. Biol. Chem.* **2007**, *282*, 8978−8983.
(19) Dafforn, T. R.; Rajendra, J.; Halsall, D. J.; Serpell, L. C.; Rodger, A. Protein fiber linear dichroism for structure determination and kinetics in a low-volume, low-wavelength couette flow cell. *Biophys. J.* **2004**, *86*, 404−410.
(20) Ardhammar, M.; Mikati, N.; Nordén, B. Chromophore orientation in liposome membranes probed with flow dichroism. *J. Am. Chem. Soc.* **1998**, *120*, 9957−9958.
(21) Rajendra, J.; Damianoglou, A.; Hicks, M.; Booth, P.; Rodger, P. M.; Rodger, A. Quantitation of protein orientation in flow-oriented unilamellar liposomes by linear dichroism. *Chem. Phys.* **2006**, *326*, 210−220.
(22) Ardhammar, M.; Lincoln, P.; Nordén, B. Invisible liposomes: Refractive index matching with sucrose enables flow dichroism assessment of peptide orientation in lipid vesicle membrane. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15313−15317.
(23) Frisch, M. J. et al. *Gaussian 03*, revision C.02; Gaussian, Inc.: Wallingford CT, 2004.
(24) The apparent rate of decolorization **1o** → **1c** is in fact the rate of thermal equilibrium establishment, that is, the sum of the rate constants for **1o** → **1c** and **1c** → **1o**.
(25) In buffer solution, and likely also in the presence of POPC liposomes, hydrolysis contributes to the overall decay of **1o** at longer times. On the contrary, no sign of hydrolysis is observed in the presence of the POPG liposomes.
(26) Wohl, C. J.; Helms, M. A.; Chung, J. O.; Kuciauskas, D. Phospholipid bilayer free volume analysis employing the thermal ring-closing reaction of merocyanine molecular switches. *J. Phys. Chem. B* **2006**, *110*, 22796−22803.
(27) Reichardt, C. Solvatochromic dyes as solvent polarity indicators. *Chem. Rev.* **1994**, *94*, 2319−2358.
(28) Rosario, R.; Gust, D.; Hayes, M.; Springer, J.; Garcia, A. A. Solvatochromic study of the microenvironment of surface-bound spiropyrans. *Langmuir* **2003**, *19*, 8801−8806.
(29) Görner, H. Photochromism of nitrospiropyrans: effects of structure, solvent and temperature. *Phys. Chem. Chem. Phys.* **2001**, *3*, 416−423.