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Nonamphiphilic Assembly in Water: Polymorphic Nature, Thread Structure, and Thermodynamic Incompatibility

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Abstract: Self-assembly of large quantities of entirely water-soluble molecules is entropically challenging. In this work, we describe the design and synthesis of water-soluble aromatic (dichromonyl) molecules that can form nonamphiphilic assemblies and the so-called chromonic liquid crystal phase in water. We discover a new molecule, 5′DSCG-diviol, that exhibits a large birefringent phase, and we show that the formation of this unique class of nonamphiphilic lyotropic liquid crystal shares enormous similarity to the polymorphism observed for crystal formation. Small-angle neutron scattering (SANS) revealed a concentration-independent rod-shaped assembly at concentrations below and above the formation of liquid crystal phase. Adding a small percentage of monoanionic aromatic molecules to the liquid crystal resulted in the elimination of the liquid crystal phase, but addition of dianionic aromatic molecules retained the liquid crystal phase. Together, these results suggest a new assembly structure for nonamphiphilic molecules in water, which is comprised of long threads of small molecules connected by salt bridges stacked over aromatic groups, with the molecular threads heavily hydrated with solvent water. Furthermore, mixing molecules with different structures can result in new liquid crystalline materials, or in segregation of the molecules into different solvation volumes, each of which contains only one type of molecule. The unusual thermodynamic incompatibility of entirely water-soluble molecules also supports the model of molecular threads, in which two polymer-like assemblies do not mix.

Introduction

Assembling a large quantity of molecules in water usually involves amphipilic molecules forming micelles, vesicles, columnar, and lamellar assemblies. These types of assemblies rely on the separation of hydrophobic and hydrophilic interactions, in which the aliphatic chains aggregate to avoid highly organized water molecules surrounding them,[1−3] and their existence were known as early as 2800 B.C. when soap-like substance were found in Babylonian clay cylinders.[4,5] Molecular assemblies in water that do not involve well-defined separation of hydrophobic and hydrophilic interactions are usually intramolecular in nature, such as protein folding, or those that involve a few copies of the molecules, such as protein dimer or oligomer formation and DNA duplex for mation. In contrast, assembling large quantities, say more than 100, of nonamphiphilic water-soluble molecules in water requires overcoming enormous entropy associated with organizing the solute and solvent molecules in space. Thus, the assembly of a large quantity of water-soluble molecules in water, without forming crystals or precipitates, can be perceived to be rare. In this work, we elucidate the possibility, the nature, and the novel assemblies of certain soluble, nonamphiphilic small molecules in water at large scale, assemblies consisting of more than $\sim 2.6 \times 10^{12}$ molecules and spanning hundreds of micrometers.[6] We demonstrate that the formation of this assembly shares enormous similarity with the occurrence of polymorphs in crystal or aggregate formation. Whereas the relations between the molecular and assembly structure for conventional thermotropic or lyotropic liquid crystals are conceivable, the relation between the molecular structure and the assembly structure for this class of nonamphiphilic molecule is not readily clear. Together with studies in small-angle neutron scattering, solvation compatibility between different molecules, and the relation between molecular structure and assembly behavior, we describe a novel assembly structure for these nonamphiphilic liquid crystal phases.

Studying such nonamphiphilic assembly will reveal how assembly at large scale (micrometers) can emerge from entirely water-soluble molecular interactions. Our interest in these nonamphiphilic assemblies originated from the discovery of a water-in-water emulsion that contains droplets of water-solvated liquid crystals.[7] This liquid crystal, discovered in the early 1970s,[8−12] is comprised of a nonamphiphilic aromatic organic salt, disodium cromoglycate (5′DSCG) (Figure 1), which exhibits high birefringence when solvated in water at concentra-

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(6) This number is estimated by the number of molecules in a uniform domain ($\sim 1500\ \mu m^2$) observed in a liquid crystal sample of 11 wt% 5′DSCG sandwiched by a 13 μm-thick spacer (See Fig. 4).

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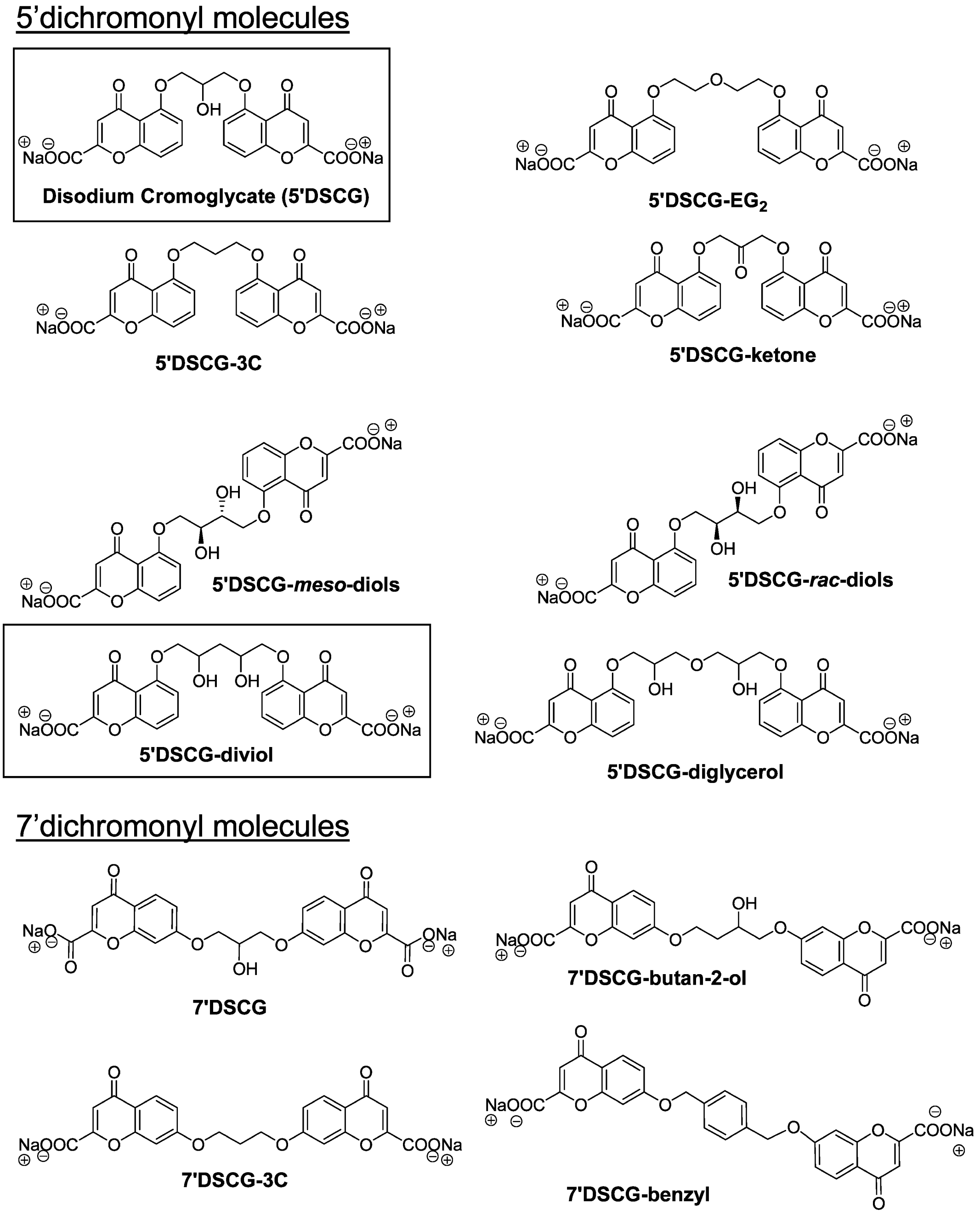

Figure 1. Dichromonyl molecules with 5′- and 7′-regiochemistry synthesized in this work. 5′DSCG-diviol is comprised of a 48% meso compounds and 52% racemic mixture, and 5′DSCG-diglycerol is comprised of a 50/50 mixture of meso and racemic compounds.

tions near and above 11 wt % at ambient temperature. With respect to the two conventional classes of liquid crystals (LCs), which consist of either organic molecules with anistropic shape for thermotropic LCs,[13,14] or amphiphilic molecules assembled in water for lyotropic LCs, this class of nonamphiphilic LCs is unique; it requires a solvent water, but the phase transition is sensitive to temperature. Because 5′DSCG is synthesized with chromonyl moiety, and because no well-defined hydrophobic and hydrophilic portions can be assigned to the structure of the molecule, historically this class of LCs is named as lyotropic chromonic liquid crystals.[11,15]

Among the molecules claimed to form chromonic LCs, however, only 5′DSCG exhibits large birefringence over the entire area of the sample (ca. 11 wt % or above of 5′DSCG in water at ambient temperature). The birefringence of 5′DSCG

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is the strongest as compared to other dye molecules claimed to exhibit chromonic LC phases. For example, nematic LC formed by 5′DSCG shows a birefringence about 200 times larger than that formed by 30 wt % Sunset Yellow dye.[16] Other dye molecules claimed to form chromonic LCs do not readily exhibit birefringence over the entire sample, but often exist as a mixture of isotropic solution and liquid crystals. No explanation has been known for such uniquely high birefringence of 5′DSCG in water. In addition, most molecules claimed to form chromonic liquid crystals are "accidental" discoveries from studying commercially available molecules.[17] The few exceptions of designed chromonic liquid crystals also exhibit weak birefringence.[18−20] Considering the scarcity of molecules (essentially none) that show such pronounced liquid crystal properties as 5′DSCG, we believe that the situation is reminiscent of the time when a single component of thermotropic liquid crystal was first discovered. As thermotropic LCs have led to an expansion in fundamental understanding and application development, we believe great opportunities lie ahead for this class of nonamphiphilic lyotropic liquid crystals.

As solvent is needed for chromonic liquid crystals, the knowledge for the solvent-free thermotropic liquid crystal, such as the dispersion force being the primary attribute for thermotropic liquid crystals, Maier−Saupe theory,[21−23] is not directly relevant; and because the mesogenic molecules are not amphiphilic, the knowledge of lyotropic liquid crystals, such as the molecular packing model developed by Israelachvili and co-workers for predicting the assembly structure from the molecular shape,[24−26] is not applicable either for this class of chromonic liquid crystals. Currently, there is no satisfactory theory describing the molecular forces and thermodynamics for the assembly formation by these nonamphiphilic molecules. We note that, due to the charges and aromatic core in the structure, one may still consider this class of molecules amphiphilic (Figure 1), in the context of having the hydrophobic and hydrophilic parts of the molecule well separated. We will elucidate in this work that being nonamphiphilic is actually critical for this class of molecules to form liquid crystal phases. Most importantly, contrary to thermotropic and lyotropic liquid crystals, of which the molecular organization is well-known, the molecular arrangement in the chromonic liquid crystals remains controversial, and essentially unsolved.[9−12,27] To elucidate the nature of this class of liquid crystals, and to discover new structures that can also exhibit such unique liquid crystal properties, we synthesized a wide range of nonamphiphilic small molecules with systematic variation in structure (Figure 1).

The understanding of the structures and forces of assemblies formed by molecules entirely soluble in water is highly significant and bears enormous potential for applications. First, understanding such assemblies will reveal how molecular organization and ordering can arise in water without involving hydrophobic/hydrophilic separation, but relying on organization of water-solvated functional groups and their interactions such as hydrogen bonding, $\pi-\pi$ stacking, salt bridge formation, and dispersion interactions. Second, this class of liquid crystals will provide a new paradigm in templated synthesis of materials[28−32] that do not require the presence of amphiphiles or surfactants, which are known to denature proteins. Realization of such highly biocompatible materials has potential applications in a wide range of disciplines including heterogeneous biocatalysis, tissue engineering, and sensor development.

Results and Discussion

Polymorphic Nature of Nonamphiphilic Lyotropic Liquid Crystals. Because 5′DSCG exhibited large birefringence over the entire sample in its LC phase, we synthesized 10 new dichromonyl molecules with systematic variations in structure and regiochemistry (Figure 1). Together with two previously reported structures, 5′DSCG and 7′DSCG,[33] these 12 molecules are categorized according to the position of the covalent linker at either the 5′ or the 7′ position of the two identical cromoglycate groups (Figure 1).

Surprisingly, small variations in molecular structure can completely abolish the formation of the LC phase for these molecules. For example, none of the molecules with a 7′-regiochemistry exhibited liquid crystals at any temperature or concentration in water. Aqueous solutions containing 7−11 wt % 7′DSCG and 7′DSCG-butan-2-ol afforded crystal/isotropic solution mixtures. 7′DSCG-benzyl and 7′DSCG-3C had extremely low solubility in water, forming noncrystalline aggregates. This result was surprising, because for other types of liquid crystals, small variations in structure of a established mesogen rarely result in complete abolishment of the LC phase but often just cause some changes in the liquid crystal properties, such as transition temperatures (for thermotropic) and molecular packing (for lyotropic).

In general, dichromonyl molecules with 5′-regiochemistry exhibited better solubility in water than those with 7′-regiochemistry. Specifically, 7′DSCG-3C does not exhibit visible dissolution in water, while 5′DSCG-3C exhibited a mixture of crystal and isotropic solution. Other 5′-dichromonyl molecules (Figure 1) exhibited different degrees of solubility in water. At ambient temperature, crystals were observed for 11 wt % 5′DSCG-ketone and 5′DSCG-rac-diols, while 11 wt % 5′DSCG-EG_2 and 5′DSCG-diglycerol were completely soluble in water

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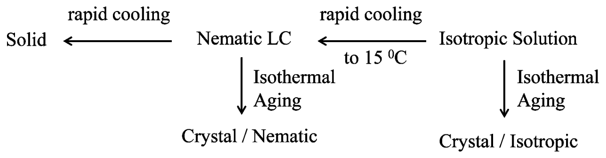

Figure 2. Assembly behavior of 5′DSCG-diviol. Isotropic solution consists of 18 wt % 5′DSCG-diviol.

and exhibited isotropic solutions under cross polars at a wide range of concentrations and temperatures.

Only one compound, 5′DSCG-diviol, which was comprised of a 48% meso compound and 52% racemic mixture (Figure 1), exhibited a liquid crystal phase similar to the known LC phase of 5′DSCG. The formation of the liquid crystal phase by this molecule shared enormous similarity to the polymorphism observed for crystal formation. Freshly prepared in water, 18 wt % 5′DSCG-diviol was an isotropic solution. Rapid cooling to 15 °C (by immersing the sample vial in ethanol followed by a stream of air on the vial) caused the formation of liquid crystal phase over the entire sample without observable precipitates or crystals. Isothermal aging by keeping the freshly prepared sample at 4 °C for 12 h gave birth to crystals, affording a mixture of LC and crystal (Figure 2). This result suggests that crystals formed by 5′DSCG-diviol appear to be more thermodynamically stable than the LC phase. Similar results were obtained for other concentrations, but with a higher temperature at the rapid cooling stage (Figure 3).

This temperature-dependent behavior for the assembly of 5′DSCG-diviol in water highly resembles the formation of polymorphs, different crystals or aggregates, formed at different conditions by the certain molecules in solid state.[34−43] A classical example is benzylamide, for which rapid cooling of a boiling solution initially results in white long needle-like crystals that turn into yellow crystals over time.[34] We note that, for 5′DSCG-diviol, crystals emerge by isothermal aging and LC phases form by rapid cooling, whereas for 5′DSCG, the LC is the only assembly observed at ambient temperature over a wide range of concentrations. On the basis of Ostwald's rule for crystals, which suggests that the least stable polymorph forms first before other more stable polymorphs emerge,[44,45] we believe that the crystal is a more stable form than liquid crystal for 5′DSCG-diviol, whereas the contrary is true for 5′DSCG. Because 5′DSCG in liquid crystal phase is known to be highly hydrated, about 260 water molecules per 5′DSCG molecule,[8] these results suggest that liquid crystals formed by the nonamphiphilic molecules (5′ DSCG and 5′DSCG-diviol) appear to be extreme cases of pseudo-polymorphism,[38−40] which describe different crystal forms of hydrates (or solvates) that may or may not have different amounts of solvent molecules.[42,43] In this nonamphiphilic LC, the positional order of the molecules is lost allowing the fluidity, but the orientational order of the hydrated molecules is retained to afford the optical properties of the crystalline birefringence. The detailed structure of the hydration and the assembly structure in these unusual LCs remain unsolved. Considering the lack of apparent driving force for the thermodynamic partitioning, the assembly structure of molecules in these LC phases is intriguing and of fundamental importance.

Current Model and Unusual Behaviors for Nonamphiphilic Lyotropic Liquid Crystals. Early studies of chromonic LC phases suggested a simple model of molecular stacks as the assembly structure (Scheme 1).[9−12,27] This model suggests that molecules stack in solution to form molecular columns driven by $\pi-\pi$ interactions of the aromatic rings. Since the discovery of this class of liquid crystal, there have been arguments and variations of this stacking model.[9−12,27] Different details include hollow columns, H-aggregates, and J-aggregates (Scheme 1).[11,46−50]

Below we describe six discovery-based experimental observations that reveal the inaccuracy of the stacking model and suggest a new molecular arrangement for these nonamphiphilic assemblies. We then describe hypotheses-driven experiments that allow further evaluation of the validity of the new and old models.

1. Nonamphiphilic Lyotropic LCs Are Extremely Sensitive to the Details of the Molecular Structure of the Mesogens, Which Cannot Be Explained by the Simple Stacking Model for Chromonic Liquid Crystals. Changing the regiochemistry of disodium cromoglycate from 5′ to 7′ while retaining the rest of the molecular structure leads to elimination of the liquid crystal phase, resulting in noncrystalline aggregate or an isotropic−crystal mixture at all temperatures. Figure 4 shows that whereas 11 wt % 5′DSCG exhibits birefringence over the entire sample, samples containing 7−11 wt % 7′DSCG (or 7′DSCG-butan-2-ol, see Supporting Information) in water showed a mixture of crystals and isotropic solution. At 7 wt %, 5′DSCG in water is an isotropic solution (Figure 4B), but 7′DSCG exhibits scattered crystals in an isotropic solution (Figure 4F and G). Molecules 7′DSCG-3C and 7′DSCG-benzyl exhibited extremely low solubility in water, and no birefringence was observed for the aggregate formed by these two molecules.

2. Birefringence of Early Assembly Reveals Drastic Differences between the Structure of LCs and Crystals Formed by Dichromonyl Molecules and Suggests a Model of Molecular Threads for the Liquid Crystal Phase. The phases of conventional lyotropic LCs comprised of amphiphilic molecules are relatively insensitive to change in temperature. However, chromonic liquid crystals formed by 5′DSCG in water are highly sensitive to temperature. A transition from partially birefringent to entirely birefringent phase is observed by lowering the

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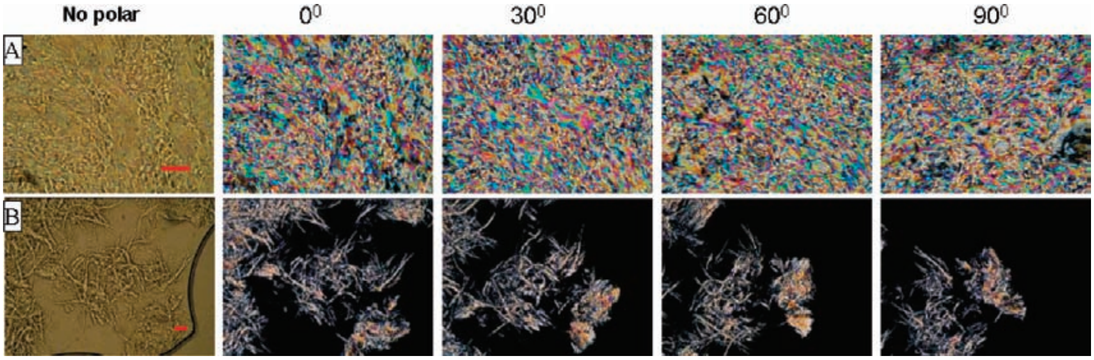
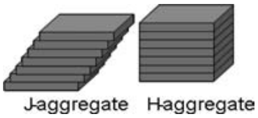


Figure 3. Optical micrographs of (A) 22 wt % 5′ DSCG-diviol in water at 19 °C (liquid crystals) and (B) crystals from 22 wt % 5′ DSCG-diviol in water after standing overnight at 21 °C. Angles of rotation of the samples under cross polars are shown above the images. Scale bar = 38 μm.

Scheme 1



ambient temperature to about 20 °C for 11 wt % 5′DSCG.[51−55] Examining the birefringence of 11 wt % 5′DSCG at the isotropic to liquid crystal transition temperature (∼20 °C) indicated that the sample did not show visible crystals, but exhibited domains of birefringence with large variation in areas that can flow and change shape (Figure 4C and D). This result is consistent with a nematic liquid crystal. For 7 wt % 7′DSCG in water, small crystals with a concentric symmetry were observed as the sample was cooled from an elevated temperature (Figure 4F and G). The shape and the size of these crystals are persistent under flow stress. Together, observations (1) and (2) suggest that the assembly structure in the LC phase of 5′DSCG is grossly different from the crystal structure formed by 7′DSCG, and a simple stacking model is unlikely sufficient for explaining both the LC and the crystal phases, although the structures between the two molecules are similar.

3. Nonamphiphilic Molecular Structure Is Needed for LC Formation of 5′DSCG. The drastic difference between the birefringence of assemblies formed by 5′DSCG and 7′DSCG suggests that connecting the linkers at 5′ positions of chromonyl moiety is important for LC formation. Keeping this regiochemistry, we synthesized a series of 5′-dichromonyl molecules with different chemical structures of the linkers. Removing the hydroxyl group in the linker of 5′DSCG (Figure 1) to afford 5′DSCG-3C severely compromised the formation of liquid crystal phases at all concentrations. Interestingly, this molecule, 5′DSCG-3C, readily exhibited a wide range of polymorphism. For example, repeated preparations of 11 wt % 5′DSCG-3C in water at ambient conditions exhibited different crystalline structures seen under cross polars (Figure 5). If one considers 5′DSCG to be amphiphilic due to the central part of the molecule being hydrophobic and the periphery of the molecule carrying two charges, then removing the central hydroxyl group should enhance the amphiphilic nature of the molecule. Furthermore, introducing two hydroxyl groups in the linker for 5′DSCG-diviol regained the LC phase. Together with the lack of LC phase by 5′DSCG-3C, these results suggest that the presence of water-soluble groups in the center of the molecule is essential for retaining the liquid crystalline phases. The simple model of molecular stacking of the hydrophobic cores of the molecules does not address the delicacy of the structural dependence for liquid crystal formation of these nonamphiphilic molecules.

4. Temporal Molecular Stacking (Suggested by NMR Spectroscopy) Does Not Necessarily Lead to Stable Assembly and LC Formation. Peak broadening of the signals in a proton nuclear magnetic resonance spectrum for a molecule often suggests a slow diffusion of molecules due to either high rigidity in the molecule, aggregation, or temporal self-association of molecules in the solution.[3] Many pharmacologically active compounds exhibit such self-association. Examples include anesthetics, antipsychotics, and antibiotics.[56−61] Observation of the peak broadening as the concentration of 5′DSCG is increased in D_2O is consistent with the formation of liquid crystals as a result of self-association or asembly.[62,63] We discovered that molecules such as 5′-monosodium cromoglycate (5′MSCG) or 5′DSCG-EG$_2$, both being water-soluble, exhibited similar peak broadening and upfield chemical shifts in their proton NMR spectra (Figures 6 and 7). However, no birefringence or sign of LC phases were observed for either of these molecules at any concentration or temperature. This result suggests that the self-association, presumably through stacking as judged by the upfield chemical shift, for molecules does not necessarily lead to chromonic LC phase. For the LC phase to emerge for nonamphiphilic molecules, we believe that the gain in free energy by self-association has to be sufficient to overcome the gain in free energy by solvation of individual molecules. However, such gain in free energy cannot be too large, otherwise crystals or precipitates will result from the self-assembly. This delicate balance of assembly and solvation likely results from the specific molecular structures in addition to stacking moieties.

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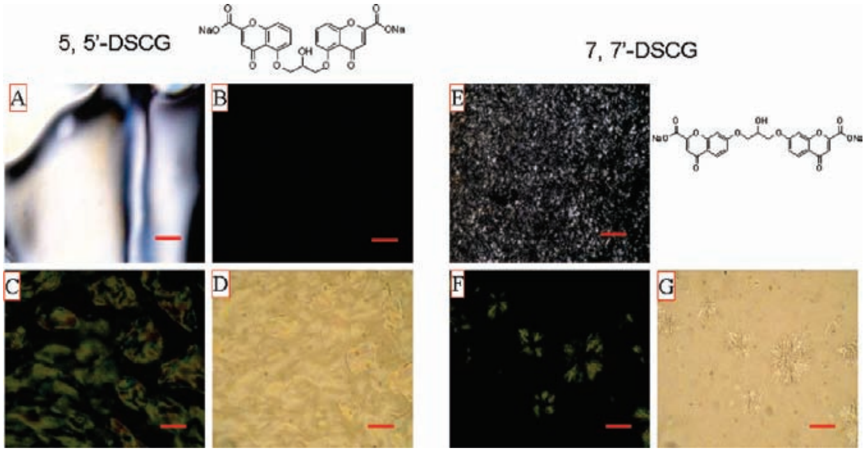

Figure 4. Liquid crystal images under cross polars of LC phase of 11 wt % 5′DSCG (A), isotropic of 7 wt % 5′DSCG (B), and isotropic/crystal mixture of 11 wt % 7′DSCG (E) at 19 °C; 11 wt % 5′DSCG at ca. 20 °C under cross polars (C) and with no polars (D); and 7 wt % 7′DSCG at ca. 20 °C under cross polars (F) and with no polars (G). Scale bar = 90 *μ*m.

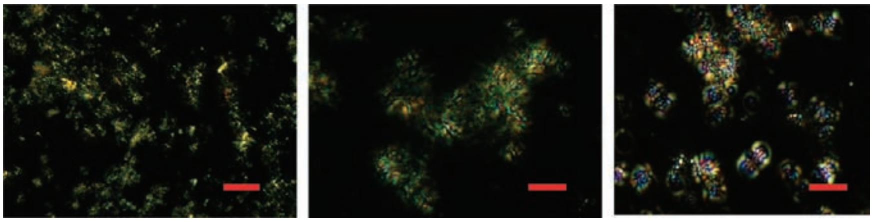

Figure 5. Crystals formed by three samples of 11 wt % 5′DSCG-3C prepared at ambient conditions. Scale bar = 38 *μ*m.

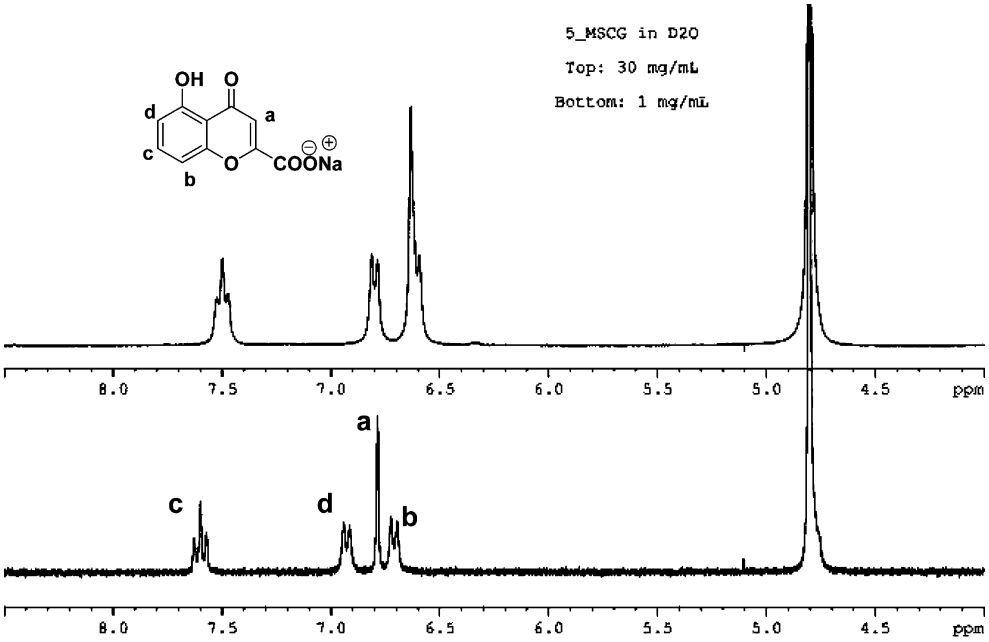

Figure 6. ^{1}H NMR spectra of 5′MSCG of different concentrations in D_2O: top, 30 mg/mL (2.76 wt %); bottom, 1 mg/mL (0.09 wt %).

5. Small-Angle Neutron Scattering (SANS) Reveals a Concentration-Independent Thin-Rod Assembly Structure. To probe directly the assembly structure of 5′DSCG, we used SANS to characterize the shape of the molecular assembly at concentrations of 5′DSCG spanning across the isotropic−LC transition (below and above 11 wt % in water). Figure 8 shows SANS of 5′DSCG in D_2O at concentrations that involve both isotropic solution (2−8 wt %) and nematic phases (10−13 wt %), which was readily observed by the emergence of the birefringence under cross polars. We note that this isotropic to LC transition at the concentration from 8 to 10 wt % is also seen in the two-dimensional scattering patterns, in which the isotropic ring (8 wt % 5′DSCG) transitioned into bimodal (10 wt % 5′DSCG) and eventually an orientated pattern (12 wt % 5′DSCG) (Figure 9). At 2 wt %, SANS of 5′DSCG did not show sign for assembly of molecules. At 6 wt % 5′DSCG, a bend was seen at q value = ~0.11 Å^{-1} in the plot of ln $I(q)$ versus ln q, which had a slope of −1 at high q values. In the nematic phase (at concentrations above 10 wt %), the anisotropic two-

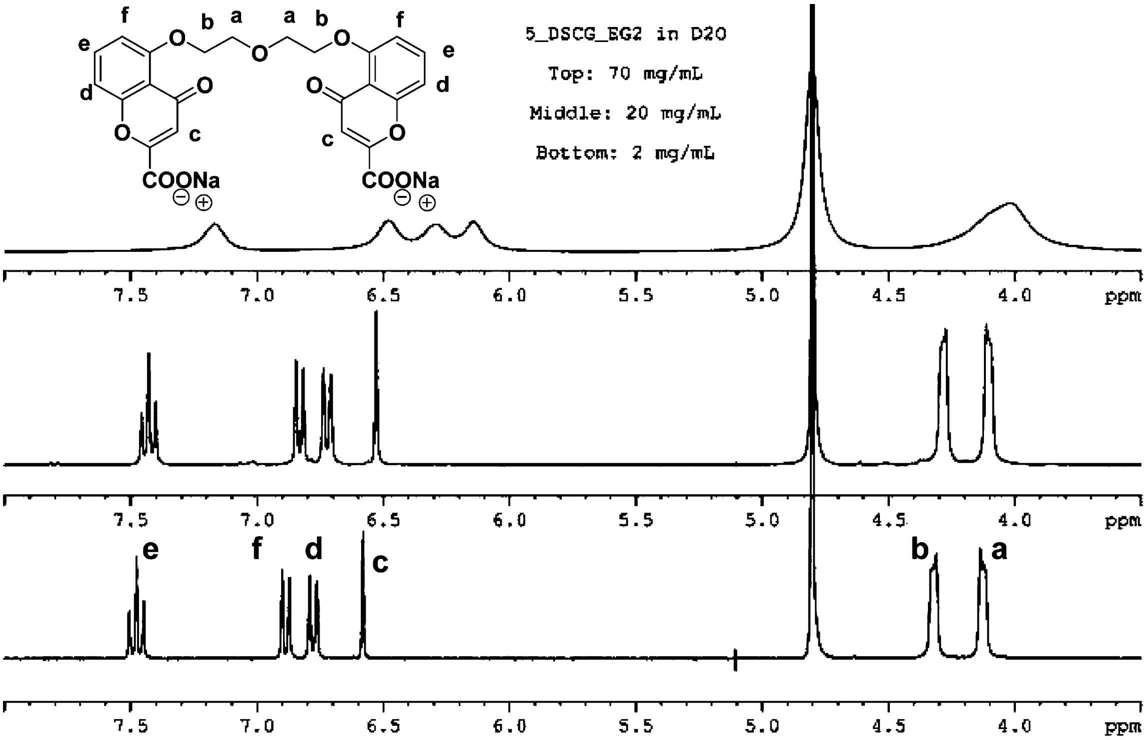

Figure 7. ^{1}H NMR spectra of 5′DSCG-EG$_2$ of different concentrations in D$_2$O: top, 70 mg/mL (6.22 wt %); middle, 20 mg/mL (1.86 wt %); bottom, 2 mg/mL (0.19 wt %).

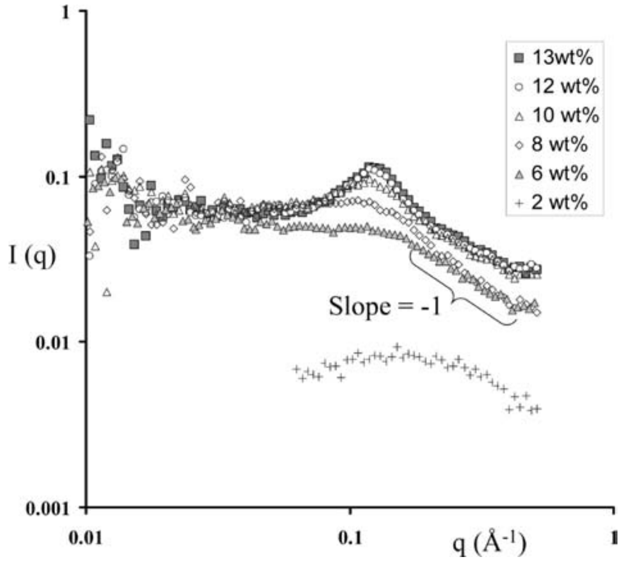

Figure 8. Small-angle neutron scattering of 5′DSCG in D$_2$O. Plot of intensity versus q (in log scale) for different concentrations of 5′DSCG shown in the graph.

dimensional SANS (Figure 9) data were also averaged isotropically to obtain the slope of the plot of ln $I(q)$ versus ln q.

This SANS result suggests several unique characteristics of the assembly. First, molecular assembly of 5′DSCG occurs at concentrations well below the emergence of the LC phase. Second, the slope of -1 for the plot of ln $I(q)$ versus ln q is characteristic of a rod-shaped assembly.[64] Third, invariance of the slope suggests that the assembly structure did not change as the concentration was increased in both isotropic and LC phase. Fourth, the bend occurring at $q = \sim-0.11$ Å^{-1} did not vastly change positions, but increased in intensity and decreased in width, suggesting that the correlated regions of LC increase in size, as the concentrations of 5′DSCG were increased from 6 to 13 wt %. This result suggests that the peak at $2\pi/q = 57 \pm 10$ Å is related to a characteristic length in the self-assembled system.

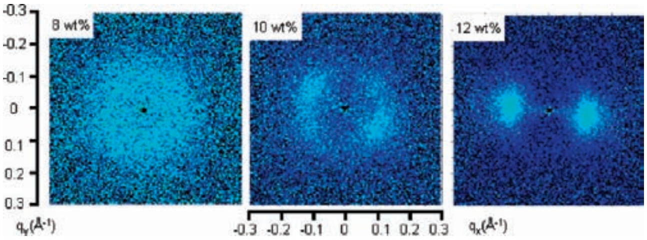

Figure 9. Two-dimensional SANS pattern of 5′DSCG in D$_2$O at 12, 10, and 8 wt %.

The SANS of 6 wt % 5′DSCG was used to check for the assembly's form factor,[65,66] $I(q) = 1 - 1/3q^2{*}R_g^2 + ...$, etc., from which the Guinier approximation (initial slope of plot $I(q)$ vs q^2)[67] gave a radius of gyration (R_g) of 17.1 Å for the assembly. As the slope at large q being -1 suggests a model of rod for the assembly, we consider the specific model for a rod of length L and negligible diameter,[66] of which $R_g^2 = L^2/12$ and gives a characteristic length of $L = 59.2$ Å for our rod form factor.[65,68]

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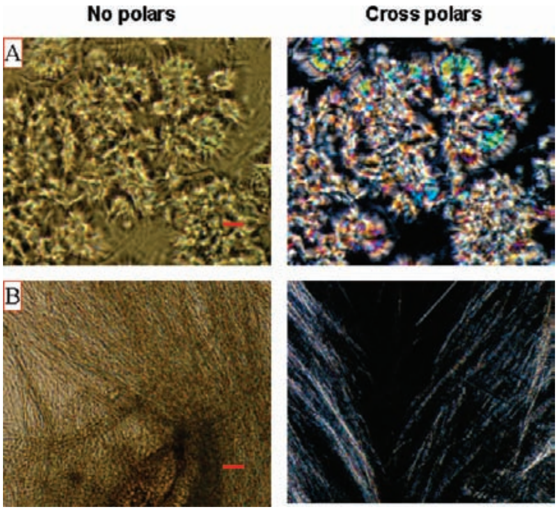

Figure 10. Optical micrographs of (A) 11 wt % 5′ DLCG in water, and (B) 11 wt % 5′ DKCG in water, all at 21 °C. Scale bar = 19 μm.

This characteristic length of the rod matches well with the length 57 ± 10 Å obtained by $2\pi/q$, where $q = \sim 0.11$ Å^{-1}. Thus, we believe that the assembly of 5′DSCG in water is best characterized by a rod-shaped assembly that has a unit of negligible cross-section diameter with an average or persistence length L around 59 Å.[69−72] Past SANS work by Raghavan and co-workers focused on high concentrations of 5′DSCG (10 wt %) in the LC phase,[49] which also exhibited the peak at $q = \sim 0.11$ Å^{-1}. The authors also characterized 5′DSCG assembly at 10 wt % using cryogenic transmission electron microscopy,[49] which gives a texture that is consistent with long rods or worm-like assemblies.[69−71] We also note that the previous stacking model for 5′DSCG,[9,10,54] which has a large cross-section area, is not consistent with the model of rods with negligible cross-section.

6. Counter Cations Are Critical for Nonamphiphilic Molecules to Form Liquid Crystals in Water. Although the liquid crystal phase for disodium cromoglycate has been known for about 38 years, the importance of the counterion sodium remains unkown. Here, we prepared dilithium cromoglycate (5′DLCG) and dipotassium cromoglycate (5′DKCG),[73] and examined their assembly in water. Interestingly, both 5′DLCG and 5′DKCG exhibited lower water solubility than 5′DSCG, but neither formed precipitates from solution. Instead, 5′DLCG and 5′DKCG form nonflowing gel-like materials at 11 wt % in solution (see Supporting Information). Examination under crossed polars reveals that, whereas 5′DLCG afforded a birefringent assembly (Figure 10A), 5′DKCG afforded fiber-like materials (Figure 10B). Such different assembly structures suggest that the counter cations, lithium, sodium, or potassium, are closely associated with the carboxylate group on the cromglycate group rather than solvated separately by water molecules.

A New Model: Threads of Molecules. On the basis of these results, we propose a new model for the molecular assembly in the nematic liquid crystal phase formed by nonamphiphilic molecules in water (Figure 11). We use 5′DSCG as a specific example to illustrate the structure of the assembly. This model consists of threads, instead of columns, of molecules that resemble a linear polymer or worm-like structure. Each thread is solvated with a hydration shell of water. When the concentration of the molecules reaches a critical value, the threads align in preferred directions to form nematic liquid crystal phases. The assembly structure inside each solvated thread consists of two or three layers of 5′DSCG molecules, which have two key characteristics. First, 5′DSCG molecules in each layer are stacked on top of the molecules in other layers in an offset and head-to-tail arrangement (Figure 11). Because each chromonyl moiety in 5′DSCG molecules consists of an electron-rich (phenolic aromatic group) and an electron-deficient ring (partially aromatic chromonyl ring), the head-to-head and the offset head-to-tail stacking modes between these aromatic rings will afford different energies (Scheme 2). Direct head-to-head stacking will have the electron-rich ring on top of the electron-rich ring, and the electron-deficient ring on top of the electron-deficient ring, which is energetically unfavorable. On the contrary, the offset head-to-tail geometry is of lower energy because of the better electrostatic match for the stacked rings.

Second, molecules in the thread are connected through salt bridges formed by the disodium carboxylate groups on each end of the molecule. The salt bridges are stacked on another aromatic ring of the chromonyl group (Figure 11). Forming salt bridges in aqueous solution is favorable due to pairwise Columbic ionic interactions, but highly unfavorable due to the requirement of desolvating ions from water solvent. For instance, salt bridges may or may not form when amino acid residues are in protein structures. However, Smithrud and co-workers demonstrate that formation of salt bridges can be greatly promoted in water by having aromatic rings within van der Waals distance of the ion pairs.[74] In addition, a high percentage of positively charged amino acid residues, such as 50% of arginines, is located within van der Waals distances of aromatic groups in a protein.[75,76] Together, these findings suggest that the aromatic rings can create a hydrophobic microenvironment for desolvating ions in close contact to facilitate salt bridge formation and provide compelling support for the proposed salt bridge in the thread model (Figure 11). We note that in addition to stacked salt bridges by the aromatic rings, the details of the solvation of the assembly in water play an important role for stabilizing such assemblies. As a consequence, the assembly structure varies enormously when the cation is changed from sodium to potassium or lithium.

Experiments (1) and (2) above suggest that the connecting position of the linker and the presence of the hydroxyl group in the linker are both important for the formation of liquid crystal. Early study reported that 5′DSCG strongly hydrates with water molecules (solid solution holds 9, and nematic phase holds up to 260 water molecules);[54] together with our finding, we believe that the hydrophilic linker is assisting the hydration of 5′DSCG to prevent precipitation of 5′DSCG, which can be driven by π stacking. We believe that, for formation of nonamphiphilic liquid crystals in water, the strong hydration (or specific water binding) is needed to prevent precipitation, but at the same time the self-association is needed to overcome

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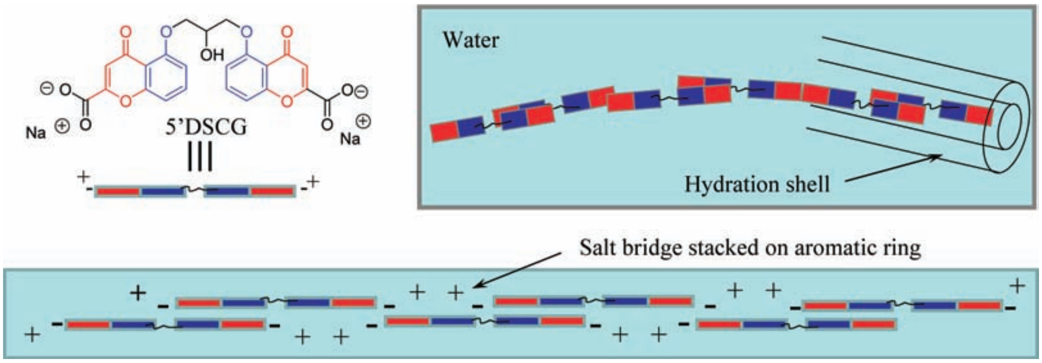
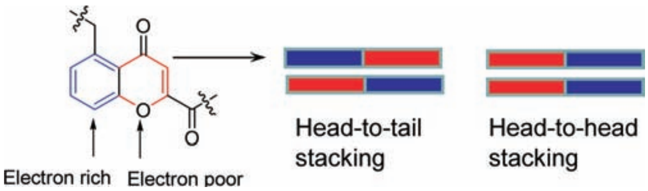


Figure 11. Proposed thread model for the assembly of nonamphiphilic 5′DSCG in water: salt bridges stacking on aromatic rings and hydration shell of 5′DSCG afford a worm or polymer-like assembly that further interacts to form liquid crystal phases.

Scheme 2



the solvation of individual molecules. The threads of molecules in this model resemble long chains of polymer with segments of certain persistent lengths with negligible cross-section area. The SANS result for 5′DSCG suggests the persistent length to be about 59 Å. Overall, this model is similar to the aggregates observed in other systems including DNA[77−79] and elongated micelles.[80,81]

Hypotheses and Validations for the Model of Molecular Threads. Mixing two or more thermotropic mesogens that are solids on their own often results in liquid crystal phases.[82] In fact, in the liquid crystal display industry, mixing different molecules is a common strategy for achieving desired liquid crystal properties including viscosity, dielectric anisotropy, and birefringence.[13,14] Likewise, mixing two different amphiphiles such as surfactants in water usually results in aggregates that may or may not bear a new assembly structure. The underlying principle for these mixing practices is that mesogens are thermodynamically compatible, so they mix instead of separating into different phases. Below, we examine whether a similar principle of thermodynamic compatibility is true for nonamphiphilic lyotropic liquid crystals, and we use the mixing strategy to test the new and old models.

For the thread model, two charges on a molecule are required to keep the molecular thread connected (Figure 11). Adding molecules bearing only one charge will break the molecular threads by terminating the salt bridges (Figure 12A). Breaking the assembly of molecules will disrupt the formation of the liquid crystals and attenuate substantially the birefringence of the sample. For the stacking model, on the contrary, the monovalent charged molecules should be able to intercalate into the stacks, and may even promote the stacking due to different polarizability of the aromatic rings (Figure 12B), and thus retain the birefringence of the sample.

Monovalent Charged Molecules Function as Thread Terminators Rather than Column Stackers. Here, we examined the effect of adding three different monovalent charged molecules, 5′-monosodium cromoglycate (5′MSCG), adenosine 3-monophospate sodium salt (AMP), and resorufin sodium salt, on the birefringence of liquid crystals formed by 11 wt % 5′DSCG in water. Figure 13 shows the optical images of 5′DSCG LC samples mixed with increasing concentration of 5′MSCG at different temperatures. As shown above (Figure 4), decreasing the temperature increases the liquid crystallinity for this class of nonamphiphilic LCs, which is revealed by the increase of observed birefringence. At low concentrations of added 5′MSCG (1.14 and 4.56 wt %) in 11 wt % 5′DSCG, the birefringence was completely eliminated at high temperature (21 °C), and only a little birefringence was retained at low temperature (18 °C). At slightly higher concentration of 6.84 wt % of added 5′MSCG, no birefringence was observed at any temperature. The few bright spots at 18 °C appeared to be crystals or precipitates that emerged from the sample. For the other two monovalent charged molecules, AMP and Resorufin sodium salt, birefringence of 5′DSCG liquid crystal samples was also eliminated (see Supporting Information). It is important to note that these aromatic molecules with monovalent charges do not merely lower the isotropic−liquid crystal transition temperature, but completely destroy the liquid crystal phases, for example, when 6.84 wt % 5′MSCG is added. Peak broadening of the proton NMR suggests that 2.76 wt % 5′MSCG is capable of self-association through stacking (Figure 6), but 1.14 wt % 5′MSCG already eliminates the birefringence of 5′DSCG liquid crystals. This result suggests that the stacking is unlikely the primary attribute for the formation of liquid crystals.

“Molecular Connector” Experiment Supports the Thread Model. Next, we examined the effect of adding dianionic molecules on the liquid crystallinity of 5′DSCG samples. For this experiment, we chose 5′DSCG-EG$_2$ because it is water-soluble and bears molecular structure similar to that of 5′DSCG. These two properties are crucial for maintaining 5′DSCG and 5′DSCG-EG$_2$ to have similar solvation and be thermodynamically compatible. Furthermore, 5′DSCG-EG$_2$ does not form liquid crystals or afford crystals even at high concentration in water. As such, the effect of divalent charges for connecting the molecules in the assembly can be studied with minimal

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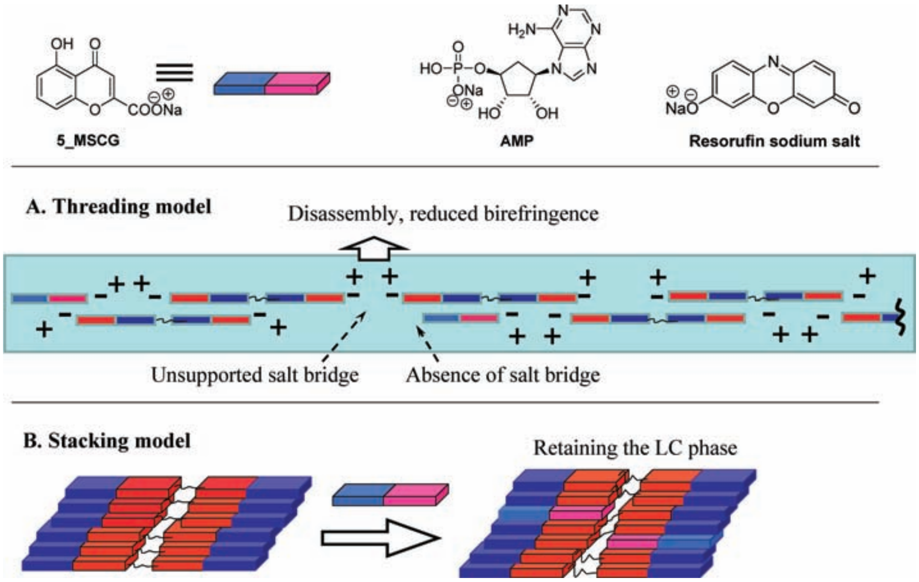

Figure 12. Hypotheses for chain terminator (A), in which 5′MSCG forms salt bridges with 5′DSCG and thus terminates the linear assembly of threads of 5′DSCG; and for column stacker (B), in which monovalent molecule (5′MSCG, AMP, or Resorufin) intercalates into the stacks of 5′DSCG.

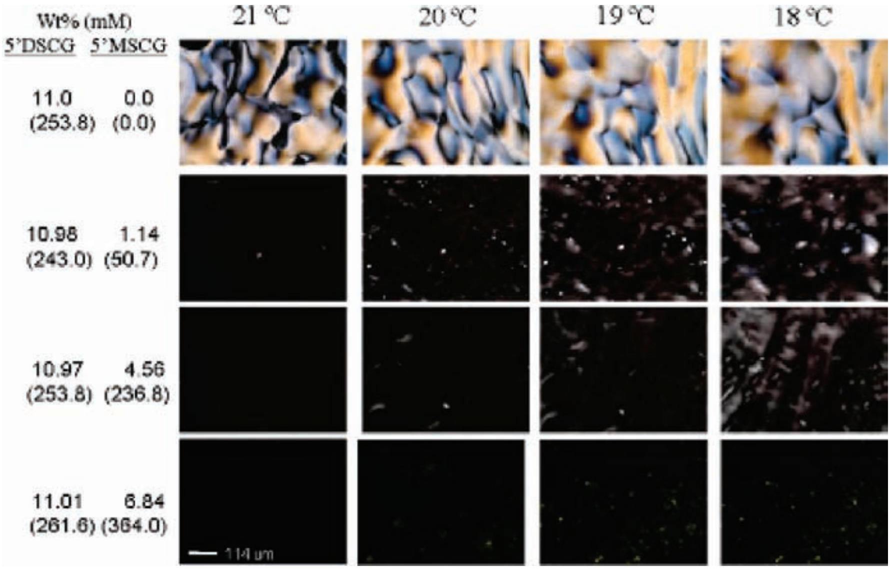

Figure 13. Optical micrographs of 11 wt % 5′DSCG mixed with different concentrations of 5′MSCG (0, 1.14, 4.56, and 6.84 wt %) in water.

influence from other factors such as phase separation or crystallization.

Figure 14 shows the optical images of 11 wt % 5′DSCG added with different concentration of 5′DSCG-EG$_2$ under cross polars. In drastic contrast to adding 5′MSCG, birefringence was observed for all samples. At lower concentration (2.28 wt %) of added 5′DSCG-EG$_2$, droplets with bright birefringence were observed. For all samples, the size of the birefringent domain increased as the temperature decreased. More importantly, the size of the birefringent domains grew as the concentration of 5′DSCG-EG$_2$ increased. This observation presented a phenomenon opposite to that observed by adding 5′MSCG, in which increasing the concentration of 5′MSCG eliminated the birefringence of the liquid crystals. Although adding 5′DSCG-EG$_2$ appeared to lower the isotropic−LC transition temperature, the liquid crystal phase was fully restored when a moderately high concentration of 5′DSCG-EG$_2$ was reached. These results support the thread model as the molecular assembly for this class of liquid crystals, in which two charged units on the molecule are needed to maintain a stable molecular assembly.

Thermodynamic Incompatibility of Nonamphiphilic Molecules. Mesogens for either of the conventional thermotropic or lyotropic LCs are usually miscible, implying that the mesogens are thermodynamically compatible in each type of LCs. However, for molecules dissolved in water, it is possible that they may not be thermodynamically compatible, and such thermodynamic incompatibility is not just limited to the “oil and water do not mix” phenomenon for surfactants. Another well-documented class of phase separations in water without well-defined hydrophobic−hydrophilic separation exists for biopolymers often observed in food sciences.[83−87]

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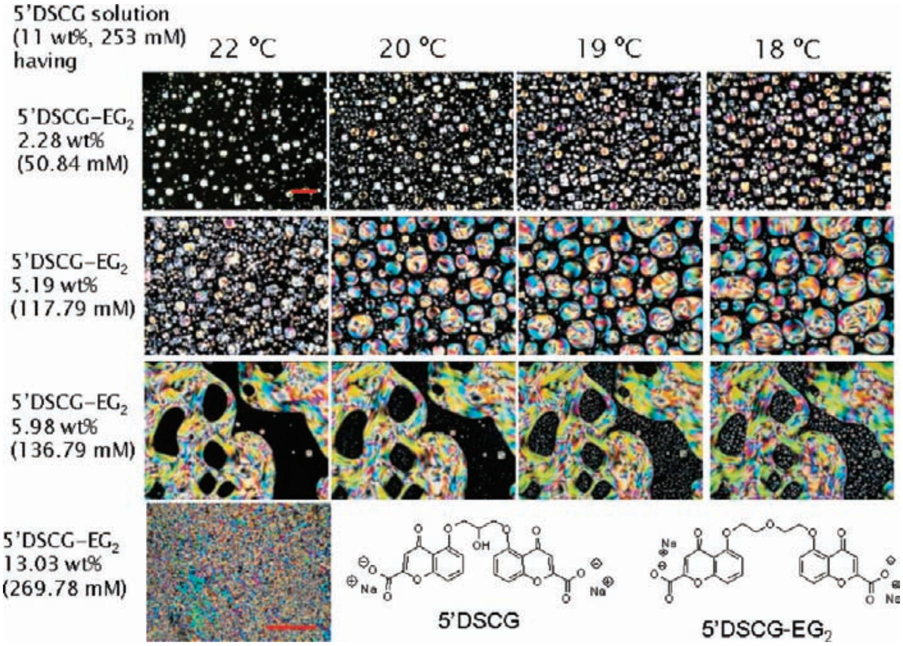

Figure 14. Optical micrographs of 11 wt % 5′DSCG having different concentrations of 5′DSCG-EG2 (2.28, 5.19, 5.98, and 13.03 wt %) in water at different temperatures. Scale bar = 38 μm.

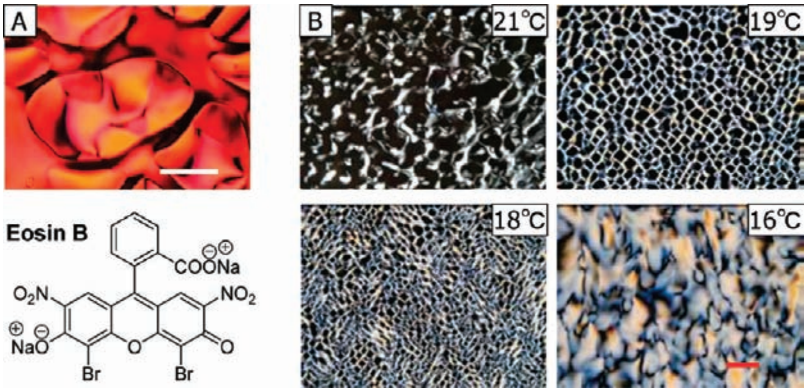

Figure 15. Optical micrographs of (A) 11 wt % (247.95 mM) 5′DSCG and 49.67 mM Eosin B at 19 °C and (B) 11.00 wt % (247.90 mM) 5-DSCG and 50 mM 5-DSCG-*rac*-diols in water. Scale bar = 38 μm.

For example, at certain concentrations, maltodextrin forms spherical water-solvated domains in a continuous aqueous solution of gelatin.[84,86,87] In this work, we discovered that nonamphiphilic small molecules can also exhibit thermodynamic incompatibility: while being water-soluble, two different molecules may not be solvated homogeneously in an aqueous solution. We made this discovery by mixing 5′DSCG with other divalently charged molecules. These molecules include Eosin B, a fluorescent red dye used in histology, 7′DSCG, 5′DSCG-*rac*-diol, 5′DSCG-*meso*-diol, and Sunset Yellow dye. Whereas 7′DSCG, 5′DSCG-*rac*-diol, and 5′DSCG-meso-diol share structures similar to that of 5′DSCG, Sunset Yellow dye is of a grossly different structure.

Mixing 5′DSCG with Eosin B, 7′DSCG, or 5′DSCG-*rac*-diol all lowered the isotropic−liquid crystal transition temperature for 11 wt % 5′DSCG several degrees below 20 °C. For example, a mixture of 11.00 wt % (~247.9 mM) 5′DSCG and 2.68 wt % (~49.7 mM) Eosin B had a transition temperature for isotropic−LC mixture to the entire LC phase at 19 °C; a mixture of 10.94 wt % (~246.1 mM) 5′DSCG and 2.26 wt % (~50.8 mM) 7′DSCG transitioned at 18 °C; and a mixture of 11.00 wt % (~247.90 mM) 5′DSCG and 2.34 wt % (~49.78 mM) 5′DSCG-*rac*-diols transitioned at 16 °C (Figure 15). Because the liquid crystal phase was fully rendered over the entire sample at lower temperatures, these results suggest that these molecules are thermodynamically compatible with 5′DSCG even though the isotropic−liquid crystal transition temperature is lowered.

Mixing 5′DSCG and 5′DSCG-*meso*-Diol Results in a New Liquid Crystalline Material. We prepared two mixtures with different compositions: 11.00 wt %/2.34 wt % and 9.03 wt %/2.48 wt % for 5′DSCG/5′DSCG-*meso*-diol. Both samples are more viscous than just 11 wt % 5′DSCG in water. Rotating under cross polars, both samples exhibited fibrous structures that were highly birefringent (Figure 16). The sample with lower concentration of 5′DSCG (9.03 wt %) exhibited a finer fibrous structure than that shown by the sample with higher concentration of 5′DSCG (11.00 wt %).

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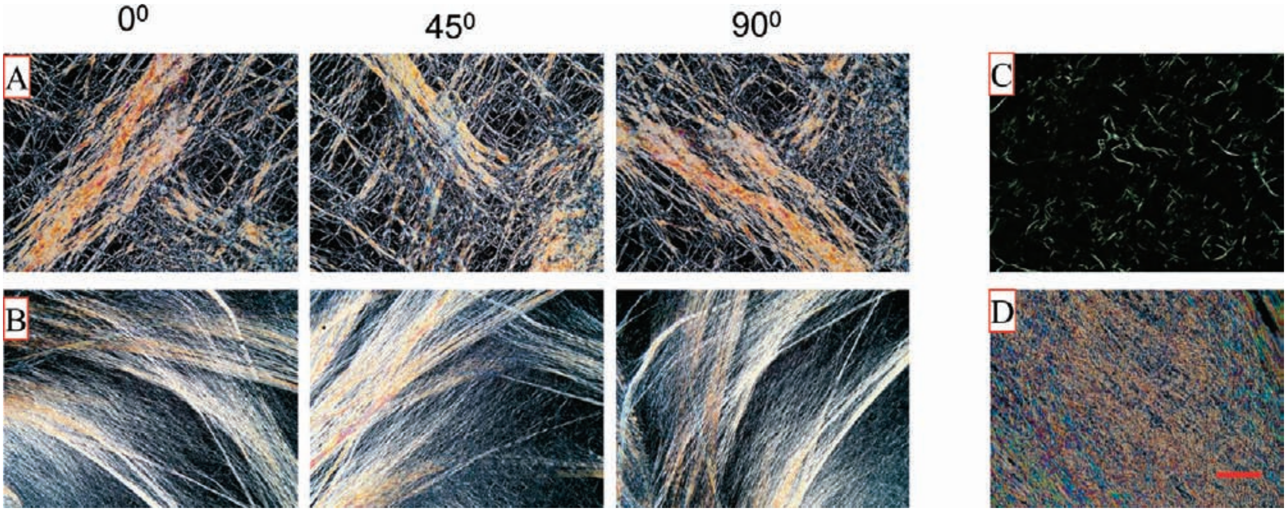

Figure 16. Optical micrographs of (A) 11.00 wt % (247.90 mM) 5′DSCG and 49.78 mM 5′DSCG-*meso*-diols in water at 21 °C; (B) 9.03 wt % (199.22 mM) 5′DSCG and 53.23 mM 5′DSCG-*meso*-diols in water at 21 °C; and (C) upper layer and (D) bottom layer after aging the sample (B) overnight. Angles of rotation of the samples under cross polars are shown above the images. Scale bar = 38 μm.

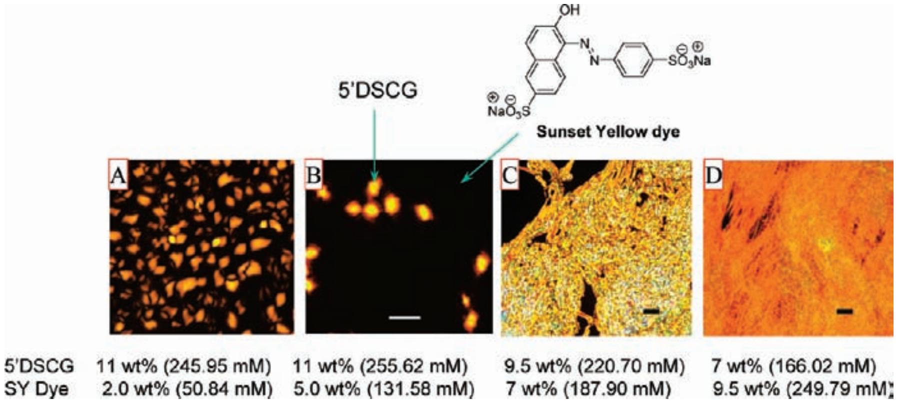

Figure 17. Optical micrographs of 5′DSCG and Sunset Yellow dye for different concentrations in water at 21 °C. Scale bar = 19 μm.

After being aged for 24 h at ambient conditions, the samples separated into two layers with the fibrous structure as sediment on the bottom and a clear solution on top. The bottom fibrous solution still flowed and exhibited a high birefringence (Figure 16D). The clear solution in the top layer contained individual fibers when examined under cross polars (Figure 16C). The fibrous layer was readily separated from the solution by decanting out the liquid part, and the fibrous layer was dried by vacuum. Characterization of the dried fiber by integration of peak intensity in proton NMR indicates that the liquid crystalline fiber contains a mixture of 5′DSCG and 5′DSCG-*meso*-diols with a molar ratio of 72:28 (see Supporting Information). This ratio is almost identical to the molar ratio for the amount of 5′DSCG and 5′DSCG-*meso*-diol used to prepare the sample, which is 79:21. This result suggests that the composition of the formed liquid crystalline fibers did not change as a result of mixing and aging, but solvent water was excluded to some extent. It is important to note that samples containing 9 wt % 5′DSCG alone at ambient temperature do not exhibit any birefringence and remain an isotropic solution. Thus, together with the proton NMR characterization, we believe that 5′DSCG and 5′DSCG-*meso*-diol are thermodynamically compatible, and a new liquid crystalline material is formed even at 9 wt % 5′DSCG.

Next, we mix 5′DSCG with another chromonic liquid crystal, Sunset Yellow dye, that is of a grossly different molecular structure. Adding 2 wt % (~50.8 mM) Sunset Yellow dye[50] largely retained the birefringence of 11 wt % 5′DSCG (Figure 17A), but increasing the amount of Sunset Yellow to 5 wt % caused the formation of small birefringent droplets surrounded by isotropic solution (Figure 17B). This result was different from the complete annihilation of liquid crystal phases by adding 5′MSCG or other monovalently charged molecules, and also different from the rendering of liquid crystal phases by adding the thermodynamically compatible molecules such as 5′DSCG-EG$_2$. Two explanations for such localized birefringence are possible. First, a new liquid crystal is formed by an assembly of a mixture of 5′DSCG and Sunset Yellow dye. Second, 5′DSCG and Sunset Yellow dye do not prefer to be homogeneously solvated in water, but the presence of Sunset Yellow dye promotes the self-association of 5′DSCG. To study the origin of these birefringent domains, we carried out two experiments. First, we lowered the concentration of 5′DSCG below the nematic liquid crystal concentration (~11 wt %) in the mixture to observe if birefringence is still retained. Second, we isolated the solution containing the birefringent phase, dried the sample, and used proton nuclear magnetic resonance to

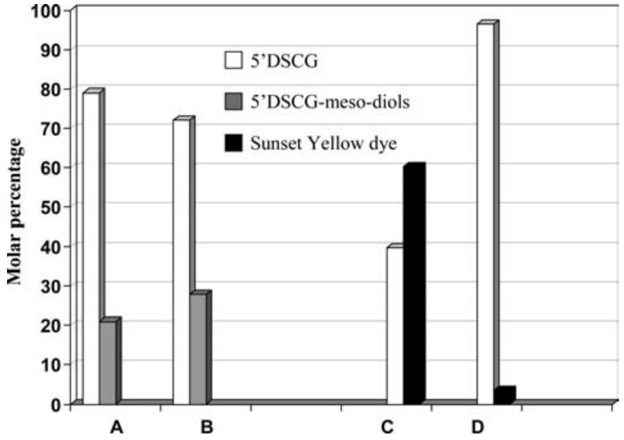

Figure 18. Mole ratio of 5′DSCG to 5′DSCG-*meso*-diol in a solution containing 9.03 wt % 5′DSCG and 2.48 wt % 5′DSCG-*meso*-diol (A), and of sediment from the same solution after aging (B). Molar ratio of 5′DSCG to Sunset Yellow dye in a solution of 7 wt % 5′DSCG and 9.5 wt % Sunset Yellow dye (C), and of the sediment from the same solution (D).

determine the composition of the molecules in the birefringent volumes. Reducing the concentration of 5′DSCG to 9.5% (with 7 wt % Sunset Yellow dye) or to 7 wt % (with 9.5 wt % Sunset Yellow dye) resulted in both samples exhibiting mixtures of strong birefringent areas and isotropic solution (Figure 17C,D). These results suggest that the presence of Sunset Yellow dye promotes the liquid crystal formation.

When the sample was aged at ambient conditions for 12 h, the sample containing 7 wt % 5′DSCG and 9.5 wt % Sunset Yellow dye settled into two layers, the top layer being a red homogeneous solution and the bottom being colorless and viscous. Upon removing the top layer by decantation, the bottom layer was dried by vacuum. Proton NMR showed that the bottom layer contained more than 96 mol % 5′DSCG with about 4 mol % Sunset Yellow dye (see Supporting Information). Compared to the ratio used for preparing the samples, which was 39.8:60.2 for 7 wt % 5′DSCG/9.5 wt % Sunset Yellow dye (Figure 18), this result indicates that 5′DSCG and Sunset Yellow dye are not thermodynamically compatible, even though both of the molecules are water-soluble. Furthermore, the presence of Sunset Yellow dye promotes the formation of liquid crystal of 5′DSCG in water.

Considering the large entropy inherent to small molecules dissolved in a solution, this partitioning of small nonamphiphilic molecules appears to be surprising. However, when the molecules assemble into a thread structure, which resembles greatly a water-soluble polymer, the thermodynamic differences between two molecules become polyvalent in nature. Thus, the collective difference between the two threads can be enormous, giving rise to a thermodynamic incompatibility similar to that for biopolymers. As such, when 5′DSCG and Sunset Yellow dye each form assemblies with its own type in water, the two assemblies behave as two different polymers or worm-like aggregates that are thermodynamically incompatible, and thus do not mix. This observation is similar to the thermodynamic incompatibility of biopolymers such as gelatin and dextran,[84,86,87] in which solvated volumes of one polymer are not miscible with the other while each polymer is dissolved in water. In contrast, the model of molecular stacking is driven mostly by the $\pi-\pi$ interactions of the aromatic moieties. Because of the favorable dipole−dipole interactions, different types of aromatic groups should have higher propensity to stack between different types of molecules rather than between the same types. As a result, we believe that the observation of thermodynamic incompatibility for these small nonamphiphilic molecules is not consistent with the stacking model.

Conclusions

Through the preparation of 10 new structures of dichromonyl molecules, we demonstrate that liquid crystals formed by nonamphiphilic molecules in water are extremely sensitive to small variations in the structural details, and that maintaining a nonamphiphilic molecular structure is necessary for the molecule to afford a liquid crystal phase. Temporal stacking of these molecules in water, as revealed by NMR peak broadening, does not necessarily lead to stable assembly or liquid crystal formation. The early assembly structure shown by the birefringence and the small-angle neutron scattering indicates that the assembly of molecules in the liquid crystal phase is of more thread-like structure rather than molecular stacks. Adding molecules with a single charge into the liquid crystal phase results in substantial attenuation or complete annihilation of the liquid crystals, whereas adding molecules with divalent charges retains the liquid crystal phases. Together, these results present a new model of molecular threads for the assembly structure in the nonamphiphilic liquid crystal in water.

We also reveal that mixing two molecules with different structures in water can result in separation of volumes, each solvating one type of molecule. This thermodynamic incompatibility from these nonamphiphilic small molecules is surprising, but supports the model of threads of molecules acting as two different incompatible polymers.[88,89] As these molecules have propensity to self-associate and be heavily hydrated with water, such thermodynamic incompatibility in water has the potential for inducing crystallization of proteins. We note that the thermodynamic incompatibility in these small molecules is consistent with the recently discovered water-in-water emulsion that involves polymer coating of water droplets,[7] which bears tremendous potential for developing amphiphile-free templated synthesis for new materials.

Experimental Section

The synthesis for 5′- and 7′-dichromonyl molecules (Figure 1) was modified from an early report.[33] The routes and characterization of all of the products were documented in the Supporting Information. The Sunset Yellow dye, Eosin B, 4-amino-1, 1′-azobenzene-3,4′-disulfonate, adenosine monophosphate sodium salt (AMP), and all other reagents were purchased (Aldrich, Fisher, TCI-America or Acros) and used as received except for Sunset Yellow dye, which was further purified by repeated precipitations. Fisherfinest glass microscope slides were purchased from Fisher Scientific. Deionized water with a resistivity of 18.2 MΩ cm was used for preparation of all of the liquid crystal samples.

Liquid Crystal Cell Assembly and Birefringence Measurement. The liquid crystal samples were assembled in a sandwiched cell composed of two glass microscope slides with one sheet of Saran Wrap (13−15 μm) to afford a spacer. The sheets of Saran Wrap were punched to create a hole to accommodate the liquid crystal sample to be sandwiched between the glass slides. The sample was subjected to vortex mixing for at least 1 min before being loaded between the slides and was sealed with binder clips on each side to prevent the evaporation of water. Samples were viewed under crossed polarizers on an Olympus BX51 polarizing

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microscope and were rotated from an arbitrary starting point to record the birefringence.

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Supporting Information Available: Design of the synthesis and characterization (H^1 and C^{13} NMR spectra and HRMS) of the synthetic products, determination of mixture composition, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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