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Authors: Lei Zhang, Chao Ma, Jing Sun, Baiqi Shao, Giuseppe Portale, Dong Chen, Kai Liu, and Andreas Herrmann

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# Genetically engineered supercharged polypeptide fluids: fast and persistent self-ordering induced by a touch

Lei Zhang<sup>†</sup>, Chao Ma<sup>†</sup>, Jing Sun, Baiqi Shao, Giuseppe Portale, Dong Chen, Kai Liu\*, Andreas Herrmann\*

Abstract: Mechanically induced disorder-order transitions have been studied in fluid surfactant solutions or polymer thermotropic liquid crystals. However, the isothermally induced ordered phases do not persist after cessation of shear, which limits their technological applicability to a great extent. Moreover, no such stimuli-responsive materials involving biomacromolecules have been reported although biopolymer liquids are gaining a lot of attention. Here we introduce a new type of biological fluid system in which anionic polypeptides are complexed with cationic surfactants. The resulting fluids exhibited very sensitive isotropic-nematic transition triggered by shear. Surprisingly, the formed liquid crystal was preserved after cessation of mechanical stimulus. Self-ordering behavior of the material was achieved through water flow and finger pressing. The latter mechanical induction resulted in the formation of complex pattern that can be read out by birefringence allowing recording fingerprint information.

Phase transitions of materials triggered by external stimuli, including application of electric fields,<sup>[1]</sup> magnetic fields,<sup>[2]</sup> light,<sup>[3]</sup> shear,<sup>[4-6]</sup> or temperature changes<sup>[7]</sup> are of great interest because structural rearrangements within the materials result in abrupt changes of material properties. Among them, shear-induced disorder-order transitions in soft polymeric materials have been extensively investigated because they are important for the optimization of processing conditions within the oil and plastic industry as well as for the function and properties of cell membranes and biological fibers in Nature.<sup>[8-14]</sup> For instance, isotropic-nematic (I-N) and isotropic-smectic (I-S) transitions were realized in amphiphile micellar solutions<sup>[8-11]</sup> and thermotropic liquid crystals (LCs)<sup>[12,13]</sup> under steady shear flow. However, it is hard to stabilize the resulting ordered phases after cessation of shear,<sup>[8-13]</sup> which may limit harnessing their

- State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 130022, Changchun, China. E-mail: kai.liu@ciac.ac.cn
- Dr. L. Zhang, C. Ma, J. Sun, Prof. G. Portale, Prof. A. Herrmann
- Zernike Institute for Advanced Materials, Nijenborgh 4, 9747 AG Groningen, The Netherlands. E-mail: herrmann@dwi.rwth-aachen.de
- Dr. L. Zhang

Prof. D. Chen

<sup>IP</sup>Present address: DWI-Leibniz Institute for Interactive Materials, Forckenbeckstr. 50, 52056 Aachen, Germany and Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Worringerweg 2, 52074, Aachen, Germany favorable properties. Therefore, maintaining an ordered state induced from an isothermal disordered phase in polymer fluids in absence of an applied shear force remains an important challenge.

Supramolecular self-assembly has attracted considerable interest for the fabrication of biomacromolecular soft materials. <sup>[15-17]</sup> For instance, a series of biopolymer LCs and disordered liquids made of nucleic acids, [18,19] polypeptides, [20,21] proteins,<sup>[22,23]</sup> and virus particles<sup>[24,25]</sup> have been reported. However, to the best of our knowledge, no biological fluid that is characterized by shear-induced disorder-order transition has been disclosed. Such a new type of stimuli-responsive soft biomaterial would be very appealing for several reasons. They would facilitate biophysical measurements for structure elucidation.<sup>[26]</sup> Cross-linking of building blocks of biological fibers in the ordered phase might result in biodegradable materials appealing mechanical properties. In biocatalysis, with structurally ordered enzymes might show improved catalytic properties on their biomacromolecular substrates. Finally, the incorporation of living systems in such type of materials might lead to dynamic LCs and control of collective microbial behavior.<sup>[27]</sup> Therefore, the development of biological fluids with mechanically induced disorder-order transition properties is an attractive goal.

Herein, we prepared a new type of peptide-based liquids, which were formed by electrostatic complexation of supercharged polypeptides (SUPs) and cationic surfactants that contain an aromatic azobenzene moiety (AZO). These protein fluids exhibit fast and persistent self-ordering behavior triggered by different external shear forces, which thus opens the opportunity for the construction of force-responsive biodevices.

Supercharged polypeptides with the dominate pentapeptide repeat motifs (GEGVP) and (GEPVE) were fabricated by recombinant DNA technology and expressed in E. Coli.[28] In these SUP sequences, V is valine, P is proline, G is glycine, and E denotes the negatively charged glutamic acid. Unfolded peptide backbones with different chain lengths and charge densities (single charge or double charge per repeat unit) were produced. The series of negatively charged SUPs was comprised of E18, EE36, E36, E72, EE108, and E144. E and EE are abbreviations for the two repeat motifs while the digit denotes the number of charges of the SUPs, e.g., E18 and EE36 have the same degree of polymerization but charge density of EE36 is double compared to E18. Thus, we successfully achieved the production of SUPs with negative charges ranging from 18, over 36, 72 and 108 to 144 (Supporting Information).

Subsequently, we synthesized a cationic surfactant containing a quaternary ammonium group and a hydrophobic alkyl chain that are separated by an aromatic azobenzene moiety (Supporting Information).<sup>[29]</sup> Both the SUPs and the oppositely charged surfactant (AZO) were combined in an aqueous solution (Figure 1A). As a result, the solution became turbid (Figure S8A) and after centrifugation an orange fluid was obtained at the bottom of the tube (Figure S8B and S8C). After separation from the

Dr. L. Zhang, Dr. B. Shao, Prof. K. Liu

Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, 266042, Qingdao, China.

Institute of Process Equipment, College of Energy Engineering, Zhejiang University, Hangzhou, 310027, China.

<sup>+</sup>L. Zhang and C. Ma contributed equally to this work

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supernatant the SUP-AZO complexes exhibited gravity-induced flow behavior at ambient conditions (Figure S8D and S8E). A quantitative component determination of the SUP-AZO complexes was carried out by nuclear magnetic resonance spectroscopy (NMR). For the E18-AZO complex, a stoichiometry of the E18 and AZO surfactant was measured to be 1:25 (i.e. ca. 1.4 AZO surfactant molecules per negative charge of the SUP) (Figure S9). This indicates that a small number of extra surfactant molecules is present in the complex.



**Figure 1.** Preparation and characterization of the mechanically responsive SUP-AZO fluids. (A) SUP fluid materials are formed by electrostatic complexation of SUPs and AZO surfactants. (B) POM analysis of the shear-induced I-N phase transition of the E144-AZO sample. The right image with nematic textures was captured after exertion of shear force. Scale bar is 100 µm. (C) SAXS analysis of the shear-induced I-N transition of the E144-AZO liquid. The broad diffraction peak at q ≈ 4 nm<sup>-1</sup> is due to the kapton, which was used for sealing of the SUP-AZO fluid samples. The inset represents the molecular packing model of the nematic phase of the SUP-AZO complex (SUPs are represented in blue, surfactant head groups in green and the hydrophobic part of the surfactant in grey).

of Further characterization the liquid material bv thermogravimetric analysis (TGA) showed that the hydrophobic SUP-AZO complexes exhibit water contents of 50-60 % (w/w) (Figure S10). The water-rich polypeptide liquid droplet could be easily transferred to a glass slide by a pipette to conduct polarized optical microscopy (POM) and conoscopy analysis. In order to avoid water evaporation, a cover glass was used and all the glass edges were sealed by a plastic film. No birefringence was observed in case of the E144-AZO fluid (Figure 1B, left image and Figure S11). The absence of any diffraction pattern as seen by small-angle X-ray scattering (SAXS) (Figure 1C, left curve; Figures S12 and S13) combined with POM and conoscopic microscopy (Figure S11) suggests that no ordered structures are present within the E144-AZO complex and no alignment was induced by the substrates. However, once a small pressing force (~200 kPa) was exerted on the glass cover slip, the sample immediately became birefringent and typical nematic textures were observed (Figure 1B, right image). The corresponding SAXS profile showed one broad diffraction peak corresponding to a d spacing of 42.0 Å, which was attributed to the average diameter of the SUP-AZO complex. Based on a rough estimation of volumes and comparison between TGA and SAXS experimental data, the mesogen is composed of hydrated SUP units of ~2.5 nm thickness separated by regions containing disordered AZO surfactant molecules of ~1.7 nm thickness. (Figure 1C, right curve). These results indicated a fast phase transition from isotropic to nematic ordering of the E144-AZO fluid material at room temperature, which was triggered by shear force. Furthermore, glass substrates with parallel rubbing were used to align the liquid sample for POM investigation. A change from dark to birefringence was observed only after an application of shear force (Figure S14). It thus suggests that this phenomenon did not originate from molecular realignment.

It should be noted that the long-range ordered lyotropic LC phase was preserved over time after removal of the external force (Figure S15). Only heating above 120°C can induce a transition from the nematic phase to the isotropic state (Figure S16). These results indicate that the shear-triggered LC phase is a true low energy state. Furthermore, it was found that the nematic SUP-AZO system reassembled into a smectic phase upon water evaporation, and a preferential alignment of the SUP-AZO complex was observed (Figure S17). Control experiments involving the SUPs complexed with another type of moiety surfactant lacking the azobenzene (i.e.. didodecyldimethylammonium bromide) did not show such selfordering behavior. Thus, the  $\pi$ - $\pi$  interactions between adjacent AZO surfactants organized around the SUP backbone might play an important role in stabilizing the induced nematic phase in the absence of an applied mechanical stimulus. Thus this new class of SUP-AZO liquid materials is unique in regard to its mechanical response due to its preserved mesophase. This is in stark contrast to other shear-triggered polymer fluid systems,[8-13] which show induced ordered phases only under shear while after cessation of shear the isotropic phases are recovered. Moreover, the isothermal I-N transition properties of the SUP-AZO fluid materials depend on the molecular weight of the SUPs. POM investigations of E36, E72, and E144 fluids revealed that the backbone lengths is reciprocal to the amount of shear needed for inducing self-ordering necessitating the application of forces of ~600, ~450, and ~200 kPa, respectively (Figure S18-S20).



*Figure 2.* Investigation of the fluid behavior of the SUP-AZO fluids by a shear rheometer. (A, B) Storage (G') and loss (G'') moduli curves as a function of time with a frequency of 1 Hz and a strain of 1. The increased store moduli with shear are marked with red dotted line. (C) POM analysis of the E144-AZO fluid material before the application of shear force. (D) POM analysis of the E144-AZO sample after applying shear for 6 min. The image was captured after cessation of shear. Scale bar is 100 µm.

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Fluid behavior of the SUP-AZO complexes was further investigated by a shear rheometer. G" was found to be higher than G' over the time course of the experiment (Figures 2A and 2B), indicating liquid-like behavior.<sup>[22]</sup> However, the values of the storage moduli increased with shearing for about 5 min of the E144-AZO liquid (Figure 2A, marked with red dotted line). Notably, POM investigation indicated a phase transition from isotropic to nematic state during this process (Figure 2C and 2D). This indicates that the shear-induced nematic structure is responsible for the increase of storage moduli. In case of E72-AZO complex, mechanical moduli are lower than for E144-AZO due to the deceased SUP molecular weight. But similar behavior was detected only after 10 min (Figure 2B). These results on the one hand suggest that the storage moduli of the fluid materials are associated with the shear-induced I-N transition. On the other, they indicate that the mechanical sensitivity of the I-N transition of the materials is determined by mechanical moduli of the materials. The higher mechanical moduli of the samples with increased molecular weight result in easier realization of I-N transition.



**Figure 3.** Investigation of water flow-induced phase transition of the SUP-AZO fluids. (A) Photograph of a simple device for detection of water flow. The E144-AZO sample was injected on a glass slide and sealed with a plastic film by double sided sticky tape. (B) POM analysis of the sample before the application of water flow. (C) Photograph of the applied water flow at a rate of 40 mL/sec (water pressure 0.35 kPa, nozzle diameter 0.8 cm) on the device containing E144-AZO material. (D) POM analysis of the formed birefringence textures of the SUP-AZO liquid (5 minutes water flushing). The image was captured after cessation of the water stream. Scale bar is 100  $\mu$ m.

To show the generality of mechanical response of the SUP-AZO fluids with regard to other external shear forces, water flow was employed to induce the I-N transition. 10  $\mu$ L of the E144-AZO complex liquid was loaded on a glass slide and sealed with a thin plastic film by double sided glue tape (Figure 3A). The primitive device gave no birefringence without water flow and the active material remained in its disordered state (Figure 3B). Upon flushing water at a flow pressure of 0.35 kPa over the thin tape (Figure 3C), an orientated LC texture was observed (Figure 3D). This indicated the successful realization of fast self-ordering behavior of the SUP-AZO complexes by a stream of water within 5 min. When the flow pressure was decreased to 0.15 kPa, no birefringence was observed (Figure S21), suggesting that the I-N phase transition is sensitive to the shear strength induced by the water flow.

Motivated by the above results we made the attempt to generate complex pattern induced by shear forces. Therefore, we applied the device described above to record fingerprint information by touching the thin tape surface with the tip of different fingers. It was found that shear-induced I-N transition took place within only 1-2 sec of pressing (~100 kPa) and the resulting pattern of birefringence textures was in agreement with the corresponding fingerprint (Figure 4A and 4B). Characteristic LC textures were collected for different fingers (Figure 4B-4D). This suggests that various fingerprint types (i.e., arch, loop and whorl) controlled the orientation of the SUP-AZO molecules and thus determined the appearing birefringence patterns of the fluid material.



**Figure 4.** Investigation of the isotropic-LC phase transition of the SUP-AZO fluids triggered by finger pressing. (A) Photograph of a simple device containing the SUP-AZO materials used for recording fingerprints. (B-D) Three fingers with different fingerprint types were applied to induce the LC phase of the E144-AZO liquid materials. Specific birefringence patterns display the different fingerprints. Scale bar is 500 µm. Due to the high magnification of the polarized optical microscope in our laboratory, only small parts of the recorded fingerprints can be shown but the whole fingerprints to illustrate the reproducibility of the fingerprint-induced birefringence textures in the SUP-AZO fluids. Note, the weak "background" birefringence in B-F originated from the plastic films.

Furthermore, two consecutive experiments were carried out to illustrate the reproducibility of finger imprint on the SUP-AZO liquid material (Figure 4E and 4F). It was demonstrated that virtually the same pattern was obtained for the same region of the fingertip. Therefore, the fast self-ordering behavior of the polypeptide soft material system triggered by external force provides a simple concept for recording of fingerprint information and individuals identification. Moreover, this strategy largely differs from reported methods for fingerprint recognition. It does not require the application of ink nor does it require, electrochemiluminescence, photoluminescence, or plasmonics as read-outs.<sup>[30,31]</sup>

A new type of mechanically responsive polymer fluids based on genetically engineered supercharged polypeptides and azobenzene surfactants has been developed. An isothermal phase transition from the isotropic liquid phase to the nematic ordered state was realized by mechanotransduction. In stark contrast to previously reported shear-induced liquid systems, the triggered nematic lyotropic LC state is maintained in the SUP-

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AZO soft materials in the absence of any applied shear force. Small external mechanical shear forces like the ones from water flows induce a phase transition in the SUP-AZO complexes enabling the recording of the resulting LC birefringence as reliable signal to distinguish different flow pressure. Moreover, active SUP-AZO complex fluid layers allow the ink-free transfer of complex pattern as obtained for fingerprints, which are translated into easily recordable birefringence read-outs. In the future, we will investigate further the mechanism of maintaining the ordered lyotropic LC phase without exerting force and we will exploit these materials to generate more complex dynamic systems responding to light, catalytic turnover or biomolecular recognition events.

#### **Supporting Information**

Protein expression, surfactant synthesis, material preparation and characterization, and additional data described in the work is available from the Wiley Online Library or from the author.

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#### COMMUNICATION



**Stimuli-responsive protein fluids:** a new type of bio-based fluids exhibiting shearinduced phase transitions have been generated by electrostatic complexation of supercharged polypeptides with aromatic surfactants. This sensitive, fast, and persistent self-ordering behaviour was exploited for water flow detection and capturing of fingerprints information by easily recordable birefringence. Lei Zhang, Chao Ma, Jing Sun, Giuseppe Portale, Kai Liu\*, Andreas Herrmann\*

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