Bio-organic hybrids of DNA, peptides and surfactants: from liquid crystals to molecular sleds
Zhang, Lei

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Chapter 2 Genetically engineered supercharged polypeptide fluids: fast and persistent self-ordering induced by a touch

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.
Chapter 2

Introduction

Phase transitions of materials triggered by external stimuli, including application of electric fields,\textsuperscript{1,2} magnetic fields,\textsuperscript{3} light,\textsuperscript{4} shear,\textsuperscript{5-9} or temperature changes\textsuperscript{10} 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.\textsuperscript{11-26} For instance, isotropic-nematic (I-N) and isotropic-smectic (I-S) transitions were realized in amphiphile micellar solutions\textsuperscript{11-20} and thermotropic liquid crystals (LCs)\textsuperscript{21-24} under steady shear flow. However, it is hard to stabilize the resulting ordered phases after cessation of shear,\textsuperscript{11-24} which may limit harnessing their 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.

In recent years, supramolecular self-assembly,\textsuperscript{27} which exploits hydrogen bonding, $\pi$-$\pi$ stacking, electrostatic forces, and van der Waals interactions has attracted considerable interest for the fabrication of biomacromolecular fluids. For instance, a series of biopolymer LCs and disordered liquids made of nucleic acids,\textsuperscript{28,29} polypeptides,\textsuperscript{30,31} proteins,\textsuperscript{32,33} and virus particles\textsuperscript{35,36} 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 biomaterials would be very appealing for several reasons. They would facilitate biophysical measurements for structure elucidation.\textsuperscript{37} Cross-linking of building blocks of biological fibers in the ordered phase might result in biodegradable materials with appealing mechanical properties. In biocatalysis, 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.\textsuperscript{38} Therefore, the development of biological fluids with mechanically induced disorder-order transition properties is an attractive goal.

Herein, we report peptide based fluids, which exhibit fast self-ordering behavior triggered by external shear forces. Protein isotropic liquids were formed by electrostatic complexation of supercharged polypeptides (SUPs) and cationic surfactants that contain an aromatic azobenzene moiety (AZO) in the aqueous phase. This 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.

**Results and discussion**

Supercharged polypeptides with the dominate pentapeptide repeat motifs (VPGEG) and (VEGEG) were fabricated by recombinant DNA technology and expressed in *E. Coli*.\(^{39}\) 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 (exact sequences are given in Table 1). We note, that for the E-series the number of repeat units is slightly larger than the number of charges due to the fact that linking units between nine charged repeats do not contain a charged residue. For the double charged EE-series the same considerations apply. Therefore, 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 (for details see Supporting Information). The chain length, charge density, and their monodisperse character were confirmed by polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), respectively (Figure S3 and S4).

Subsequently, we synthesized a known cationic surfactant containing a quaternary ammonium group and a hydrophobic alkyl chain that are separated by an aromatic azobenzene moiety.\(^{40}\) (Supporting Information). 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 supernatant the SUP-AZO complexes exhibit 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 SUPs) (Figure S9). This indicates that a small number of extra surfactant molecules is present in the complex.
Table 1. General information (the name, isoelectric point, primary structure, and molecular weight) of SUPs used in this work.

<table>
<thead>
<tr>
<th>Name of SUPs</th>
<th>Isoelectric point (PI)</th>
<th>Formula</th>
<th>Molecule Weight (Da)</th>
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<tr>
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<td>GAGP[(GVGVP)(GEGVP),GWPH]</td>
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<tr>
<td>EE36</td>
<td>3.78</td>
<td>GQ[(GVGEPVEGEPGE)2GWPH]</td>
<td>11063</td>
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<tr>
<td>E36</td>
<td>3.78</td>
<td>GAGP[(GVGVP)(GEGVP),GWPH]</td>
<td>18922</td>
</tr>
<tr>
<td>EE108</td>
<td>3.27</td>
<td>GQ[(GVGEPVEGEPGE)2GWPH]</td>
<td>30442</td>
</tr>
<tr>
<td>E72</td>
<td>3.45</td>
<td>GAGP[(GVGVP)(GEGVP),GWPH]</td>
<td>36512</td>
</tr>
<tr>
<td>E144</td>
<td>3.14</td>
<td>GAGP[(GVGVP)(GEGVP),GWPH]</td>
<td>71430</td>
</tr>
</tbody>
</table>

Further characterization of the liquid material by thermogravimetric analysis (TGA) showed that the hydrophobic SUP-AZO complexes exhibit water contents of 50–60% (w/w) (Figure S10). Due to its low viscosity, the water-rich polypeptide liquid droplet could be easily transferred to a glass slide by a pipette to conduct polarizing optical microscopy (POM) 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). The absence of any diffraction pattern as seen by small-angle X-ray scattering (SAXS) (Figure 1C, left curve; Figure S11-S12) combined with POM suggests that no ordered superstructures are present within the E144-AZO complex. However, once a small force 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 order of the E144-AZO fluid material at room temperature, which was triggered by shear force. It should be noted that the long-range ordered lyotropic LC phase was preserved over time after removal of the external force (Figure S13). Only heating above 120°C can induce a transition from the nematic phase to the isotropic state (Figure S14). These results indicate that the shear-triggered LC phase is a true low
Genetically engineered supercharged polypeptide fluids: fast and persistent self-ordering induced by a touch 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 S15). Control experiments involving the SUPs complexed with another type of surfactant lacking the azobenzene moiety (i.e., didodecyldimethylammonium bromide) showed no

Figure 1. Preparation and characterization of the mechanically responsive SUP-AZO fluids. (A) SUP fluid materials are formed by electrostatic complexation of genetically engineered supercharged polypeptides and AZO surfactants. (B) POM analysis of the shear-induced disordered-ordered phase transition of the SUP-AZO fluids (here 5 μL of the E144-AZO liquid was use). The right image with nematic textures was captured after exertion of shear force. Scale bar is 100μm. The preserved LC birefringence suggested that the induced ordered phase is stable in the SUP-AZO fluid system. (C) SAXS analysis of the shear-induced isotropic-nematic transition of the E144-AZO complex liquid. The formed nematic mesophase shows an average distance of 4.2 nm. The broad diffraction peak at \( q \approx 4 \text{ nm}^{-1} \) 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 mesophase of the SUP-AZO complex (SUPs are represented in blue, surfactant head groups in green and the hydrophobic part of the surfactant in grey).
such self-ordering behaviors. Thus, the π-π 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,11-24 which show induced ordered phases only under shear while after cessation of shear the isotropic phases are recovered.

It is also noteworthy that the isothermal I-N transition properties of the SUP-AZO fluid materials depend on the molecular weight of the SUPs. The E18-AZO liquid did not undergo a shear-induced phase transition (Figure S16A and 16B). However, when the charge density was increased, as in case of double charged EE36, obvious birefringence textures were observed after application of shear force (Figure S16C and 16D). Similarly, the liquid composed of EE108-AZO exhibited an I-N transition (Figure S17). This indicates that in case of low molecular weights the charge density needs to be increased to reach a phase transition. Furthermore, POM investigations of E36, E72, and E144 fluids demonstrated that SUPs with increased backbone lengths more easily form shear-induced self-ordering necessitating the application of less force (Figure 1B and Figure S18).

To further investigate shear-induced I-N transition of the SUP-AZO complex fluids, a shear strain controlled rheometer with two stainless steel fixtures was used (Figure 2A).41 Three SUP-AZO fluids including E144-AZO, E72-AZO, and E36-AZO were prepared and sealed in plastic bags (5 × 5 mm) for the experiments. The encapsulation during these experiments was necessary to avoid water evaporation. Water evaporation was also the reason that prevented determination of the viscosity of the different SUP-AZO fluids. The three SUP-AZO fluids were disordered liquids before the application of shear force (Figure 2B). When the SUP-AZO liquid samples were placed between two opposing steel teeth (5 × 5 mm) and subjected to oscillatory shear different phase transition kinetics were observed. E144-AZO exhibited a fast phase transition from isotropic liquid phase to the nematic ordered LC state within only 5 minutes and homogeneous birefringence textures were maintained after removal of shearing (Figure 2C). In contrast, E72-AZO liquid showed shear-induced I-N transition after 10 minutes (Figure 2D). In case of E36-AZO complex, first signs of weak LC ordering within small domains were detected after 13 minutes (Figure S19). These results suggest that the mechanical sensitivity of the I-N transition of the fluid materials can be controlled by the length of the SUP backbones.
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**Figure 2.** Investigation of the relation between molecular weight of the SUPs and the shear-induced phase transition of the SUP-AZO fluids. (A) Schematic representation of the tooth rheometer to employ oscillatory shear on the SUP-AZO liquid samples. (B) POM analysis of the SUP-AZO fluid material before the application of shear force (here E144-AZO as an example). The inset shows the SUP-AZO liquid sample which was loaded and sealed in a soft plastic bag for shear studies. (C, D) POM analysis of the SUP-AZO fluid samples after the application of shear force at a fixed frequency (1Hz) and strain (4.9); (C, E144-AZO after 5 minutes shearing; D, E72-AZO after 10 minutes shearing). The images were captured after cessation of shear. Scale bar is 100 μm. Note, the weak birefringence in B was originated from the used plastic film.

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. One droplet 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 rate of 40 mL/s over the thin tape, an orientated LC texture was observed (Figure 3C and 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 rate was decreased to 20 mL/s, no birefringence was observed (Figure S20), 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 seconds of pressing 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. Furthermore, two consecutive
experiments were carried out to illustrate the reproducibility of finger imprint on the SUP-AZO liquid material (Figure 4E-4F).

![Figure 4](image-url)

**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 complexes as active materials used for recording fingerprints. (B-D) Three fingers with different fingerprint types were applied to induce the LC phase of the SUP-AZO liquid materials (here E144-AZO as an example). 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 cannot be visualized in a single image. (E, F) Two consecutive experiments were carried out to illustrate the good reproducibility of the fingerprint-induced birefringence textures in the SUP-AZO fluids. Note, the weak “background” birefringence in B-F were originated from the used plastic films.

It was demonstrated that virtually the same pattern were obtained for the same region of the finger tip. 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.42,43

Conclusions
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. The charge density and molecular weight of the monodisperse SUPs play an important role in controlling the self-ordering behavior. In stark contrast to previously reported shear-induced liquid systems, the triggered nematic lyotropic LC state is maintained in the SUP-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 and allows distinguishing different flow rates. Moreover, active SUP-AZO complex fluid layers allow the ink-free transfer of complex pattern as obtained for finger prints, 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.

Experimental section
Materials
4-octaniline, phenol, 1,5-dibromoethane and trimethylamine solution (4.2 M) were obtained from Sigma-Aldrich (Netherlands). All the starting compounds for the synthesis of AZO surfactant were used without further purification. All biochemicals for cloning and SUP expression were used as received without any further purification. The pUC19 cloning vector, restriction enzymes, and GeneJET Plasmid Miniprep kit were purchased from Fermentas (St. Leon-Rot, Germany). Digested DNA fragments were purified using QIAquick spin miniprep kits from QIAGEN, Inc. (Valencia, CA). E. coli XL1-Blue competent cells for plasmid amplification were purchased from Stratagene (La Jolla, CA). Oligonucleotides for sequencing were ordered from Sigma-Aldrich (St. Louis, MO). Alpha-cyano-4-hydroxycinnamic acid was used as matrix during mass spectrometry and was
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purchased from Thermo Scientific (US). Ultrapure water with a resistivity of ca. 18.2 MΩ·cm was used for all experiments. Other solvents used in the work were analytical grade.

**Molecular cloning and SUPs expression**

**Cloning/Gene oligomerization**

The building blocks of the SUP genes were ordered from Integrated DNA Technologies (Iowa, USA). Gene sequences and respective amino acid sequences of monomers (E9 and EE18) are shown in Figures S2a and S2b. The SUP gene was excised from the pCloneJET vector by restriction digestion and run on a 1% agarose gel in TAE buffer (per 1L, 108 g Tris base, 57.1 mL glacial acetic acid, 0.05 M EDTA, pH 8.0). The band containing the SUP gene was excised from the gel and purified using the QIAGEN spin column purification kit. pUC19 was digested with EcoRI and HindIII and dephosphorylated. The vector was purified by agarose gel extraction following gel electrophoresis. The linearized pUC19 vector and the SUP-encoding gene were ligated and transformed into chemically competent DH5α cells (Stratagene, Texas, USA) according to the manufacturer’s protocol. Cells were plated and colonies were picked and grown overnight in LB medium supplemented with 100 µg/mL Ampicillin, and plasmids were isolated using the GenElute Plasmid Miniprep Kit (Sigma-Aldrich, Missouri, USA). Positive clones were verified by plasmid digestion with PflMI and BglII and subsequent gel electrophoresis. The DNA sequence of putative inserts were further verified by DNA sequencing (GATC, Konstanz, Germany). Gene oligomerization, known as Recursive Directional Ligation (RDL), was performed as described by Chilkoti and co-workers. In brief, monomer E9 or EE18 were digested using PflMI and BglII from parent vector as one insert. A second parent vector with E9 or EE18 was cut with PflMI only and dephosphorylated afterwards as one host vector. Ligation between the insert fragment and the host vector was performed with the presence of T4 ligase at 22 °C for 1 hour. By verifying positive clones and subsequently mini-preparing of constructs, a doubled SUP fragment (i.e., E18 or EE36 in this case, shown in Figures S2c and S2d) was cloned. For dimerizing E18 to E36, E36 to E72 and E72 to E144, a similar protocol was applied. Regarding the construction of EE108, two identical fragments of EE36 were ligated to form insert EE72, which was introduced into the vector containing EE36. Notably, one valine residue per ten pentapeptide repeats was inserted instead of a glutamic acid because the recognition sites of enzymes PflMI and BglII had to be preserved during the RDL process.
Figure S1. Gene fragments and corresponding polypeptide sequences of SUP E9 (a, monomer, containing 9 glutamic acids), SUP EE18 (b, monomer, containing 18 glutamic acids), SUP E18 (c, dimer, containing 18 glutamic acids) and SUP EE36 (d, dimer, containing 36 glutamic acids). Restriction sites flanking the inserted gene are PflMI and BglII.

Expression vector construction

The expression vector pET25b(+) was modified by cassette mutagenesis for incorporation of a unique SfiI recognition site and an affinity tag consisting of six histidine residues at the C-terminus.
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(Shown in Figure S2), as described before. Briefly, the modified pET25b(+) expression vector was digested with SfII, dephosphorylated and purified using a micro-centrifuge spin column kit. The repetitive SUP gene was excised from the pUC19 cloning vector using PflMI and BglII, and purified by agarose gel extraction following gel electrophoresis. The linearized vector and SUP-encoding gene were ligated with T4 ligase (Thermo Scientific, Massachusetts, USA), transformed into DH5α competent cells and screened as described above. The constructs of pET25b-SUP were verified first by EcoRI and NdeI digestion and then sent for DNA sequencing.

**SUP expression and purification**

E.coli BLR (DE3) cells (Novagen) were transformed with the pET25b expression vectors containing the respective SUP genes. For polypeptide production, Terrific Broth medium (for 1 L, 12 g tryptone and 24 g yeast extract) enriched with phosphate buffer (for 1 L, 2.31 g potassium phosphate monobasic and 12.54 g potassium phosphate dibasic) and glycerol (4 mL per 1 L TB) and supplemented with 100 µg/mL ampicillin, was inoculated with an overnight starter culture to an initial optical density at 600 nm (OD600) of 0.1 and incubated at 37°C with orbital agitation at 250 rpm until OD600 reached 0.7. Polypeptide production was induced by a temperature shift to 30°C. Cultures were then continued for additional 16h post-induction. Cells were subsequently harvested by centrifugation (7,000 x g, 30 min, 4°C), re-suspended in lysis buffer (50 mM sodium phosphate buffer, pH 8.0, 300 mM NaCl, 20 mM imidazole) to an OD600 of 100 and disrupted with a constant cell disrupter (Constant Systems Ltd., Daventry, UK). Cell debris was removed by centrifugation (25,000 x g, 30 min, 4°C). Polypeptides were purified from the supernatant under native conditions by Ni-sepharose chromatography. Product-containing fractions were pooled and dialyzed against ultrapure water and then purified by anion exchange chromatography using a Q HP column (buffer A: 50 mM sodium phosphate, 50 mM NaCl, pH 7.4; buffer B: 50 mM sodium phosphate, 2 M NaCl, pH 7.4). Product-containing fractions were dialyzed extensively against ultrapure water. Purified products were frozen in liquid nitrogen, lyophilized and stored at -20°C until further use.

**SUP Characterization**

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<th>NdeI</th>
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<th>EcoRI</th>
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<td>MGA GPG WP HHH HHH HHH HHH</td>
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**Figure S2.** Insert sequence of modified expression vector pET-25b (+)-SfiI-H6. The vector contains a unique SfiI recognition site for inserting ELP genes and a sequence encoding for a hexa-histidine (H6) tag at the C-terminus of the expressed protein for affinity chromatography purification.
The concentrations of the purified polypeptides were determined by measuring absorbance at 280 nm using a spectrophotometer (Spectra Max M2, Molecular Devices, Sunnyvale, USA). Product purity was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel. Afterwards, gels were stained with Coomassie staining solution (40% methanol, 10% glacial acetic acid, 1 g/L Brilliant Blue R250). Photographs of the gels after staining were taken with a LAS-3000 Image Reader (Fuji Photo Film GmbH, Dusseldorf, Germany). The resulting stained gel is in Figure S3. The supercharged polypeptides exhibit different electrophoretic mobilities according to their charge and molecular weight.

**Figure S3.** SUP samples characterized by SDS-PAGE. M, PageRuler plus prestained protein ladder. Lane 1-6: EE36, E18, E36, EE108, E72 and E144. The electrophoresis behavior of those proteins are different from neutral proteins because of many negative charges.

**Mass Spectrometry**

Mass spectrometric analysis was performed using a 4800 MALDI-TOF/TOF Analyzer in the linear positive mode. The polypeptide samples were mixed 1:1 v/v with α-cyano-4-hydroxycinnamic acid matrix (100 mg/mL in 70% ACN and 0.1% TFA). Mass spectra were analyzed with the Data Explorer V4.9 (shown in Figure S4). Values determined by mass spectrometry are in good agreement with the masses that are calculated based on the amino acid sequence (shown in Table S1).
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**Figure S4.** MALDI-TOF mass spectra of supercharged polypeptides.

<table>
<thead>
<tr>
<th></th>
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<th>M ms#(Da)</th>
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<tr>
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<tr>
<td><strong>E144</strong></td>
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**Table S1.** Mass determination of SUP variants.* Represents the average molecular weight calculated with ProtParam program. # Represents molecular weight determined by MALDI-TOF mass spectrometry.

**Synthesis of AZO surfactant**

The cationic surfactant containing an azobenzene unit was synthesized in three steps. The synthesis was inspired by procedures described for similar azobenzene derivatives. 47, 56 First, azocoupling of alkylaniline with phenol was performed, followed by alkylation of the phenol with dibromoethane. Finally, quaternionization with the help of trimethylamine was carried out.
Chapter 2

Schematic for the synthesis of AZO surfactant

Step 1: synthesis of 4-((4-octylphenyl)diazenyl)phenol (1)

4-octylaniline (2.5 g, 12.2 mmol) was dissolved in a mixture of diluted HCl (3 M, 25 mL) and ethanol (25 mL), which was stirred in an ice bath. To this solution, 10 mL cold NaNO₂ (aq) (0.894 g, 13 mmol) was added dropwise and stirring was continued for half an hour. Then the solution of diazonium salt intermediate was added dropwise to a aqueous solution (15 ml) containing phenol (1.15 g, 12.2 mmol) and NaOH (0.96 g, 24.4 mmol) at 0 °C. After stirring for overnight, the resulting solution was neutralized by HCl. The precipitate was filtered off, washed with water and dried. The product was purified by column chromatography on silica gel with solvent (hexane : chloroform = 1:5). After purification, a yield of 88% was determined. ¹H NMR (400 MHz, CD₃OD): δ 7.81-7.72 (m, 4 H), 7.33-7.28 (m, 2 H), 6.93-6.87 (m, 2 H), 2.70-2.64 (m, 2 H), 1.66-1.64 (m, 2 H), 1.36-1.27 (m, 10 H), 0.92-0.86 (m, 3 H). ¹³C NMR (100 MHz, CDCl₃): δ 158.4, 150.9, 147.1, 146.2, 129.2, 124.9, 122.7, 116.0, 36.0, 32.0, 31.4, 29.6, 29.4, 22.8, 14.3.
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Figure S5. NMR analysis of compound 1. a) $^1$H-NMR spectrum of 1 (25°C, 400 MHz, CD$_3$OD). b) $^{13}$C-NMR spectrum of 1 (25°C, 100 MHz, CDCl$_3$).

Step 2: synthesis of 1-(4-(2-bromoethoxy)phenyl)-2-(4-octylphenyl)diazene (2).

The mixture of compound 1 (1g, 3.2 mmol), 1,2-dibromoethane (32 mmol), and K$_2$CO$_3$ (0.88 g, 6.4 mmol) was slowly dissolved in 35 ml ethanol under magnetic stirring. Then the solution was heated to 80°C and refluxed for 24 hours. After reaction, the solvent was removed under reduced pressure and then the crude product was redissolved in dichloromethane. After filtration, some undissolved impurities were removed. Finally, the crude product was purified by column chromatography on silica gel (hexane : chloroform = 1:2) with a yield of ca. 69%. $^1$H NMR (400 MHz, CDCl$_3$): δ 7.91 (dd, J=9.2 Hz, 2.8 Hz, 2 H), 7.82 (dd, J=8.4 Hz, 2.4 Hz, 2 H), 7.31 (dd, J=8.4 Hz, 2.4 Hz, 2 H), 7.02 (dd, J=9.2 Hz, 2.8 Hz, 2 H), 4.35 (t, J=6.4 Hz, 2 H), 3.66 (t, J=6.4 Hz, 2 H), 2.68 (t, J=7.6 Hz,
2 H), 1.66 (t, J=7.6 Hz, 2 H), 1.38-1.28 (m, 10H). 0.98-0.85 (m, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 160.3, 151.0, 147.6, 146.2, 129.2, 124.7, 122.7, 115.0, 68.1, 36.0, 32.0, 31.5, 29.6, 29.4, 28.9, 22.8, 14.3.

Figure S6. NMR analysis of compound 2. a) $^1$H-NMR spectrum of 2 (25°C, 400 MHz, CDCl$_3$). b) $^{13}$C-NMR spectrum of 2 (25°C, 100 MHz, CDCl$_3$).

Step 3: synthesis of N,N,N-trimethyl-2-((4-octylphenyl)diazonethyl)phenoxy)ethan-1-ammonium bromide (3).

Product 2 (0.34 g, 0.8 mmol) was dissolved in 25 ml ethanol and then trimethylamine (4.2 M, 1.9 ml) was added. The solution was refluxed at 80°C for 24 h. After reaction, solvent was removed under reduced pressure. Crude product was redissolved in water, filtered and dried to give product 3 (yield: 74%). $^1$H NMR (400 MHz, CDCl$_3$): δ 7.90 (d, J=8.4 Hz, 2 H), 7.79 (d, J=8.0 Hz, 2 H), 7.29 (d, J=7.6 Hz, 2 H), 7.04 (d, J=8.4 Hz, 2 H), 4.59 (s, 2 H), 4.36(s, 2 H), 3.62 (s, 9 H), 2.66 (t, J=8.0 Hz, 2 H), 1.62 (t, J=7.8 Hz, 2 H), 1.32-1.27 (m, 10H). 0.87 (t, J=6.8 Hz, 3 H). $^{13}$C NMR (100 MHz,
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CDCl$_3$): δ 159.2, 150.8, 147.8, 146.3, 129.1, 124.8, 122.8, 115.2, 65.3, 62.9, 55.2, 36.0, 32.0, 31.4, 29.6, 29.5, 29.4, 22.8, 14.2.

Figure S7. NMR analysis of compound 3. a) $^1$H-NMR spectrum of 3 (25°C, 400 MHz, CDCl$_3$). b) $^{13}$C-NMR spectrum of 3 (25°C, 100 MHz, CDCl$_3$).

**Preparation of the SUP-AZO complex fluids**

An aqueous solution of SUP with a concentration of ~220 μM (E18, EE36, EE108, E36, E72, and E144)) was obtained by dissolving lyophilized SUPs in 100 mM NaCl solution. In a second solution made from ultrapure water, the concentration of AZO surfactant was adjusted to 10-20 mM at room temperature. Both solutions were combined in a ratio so that ~2.5 mol of surfactant equal 1 mol of glutamate residues within the SUP. As a result of mixing, the yellow solution became cloudy because the hydrophobic SUP-AZO complex segregated from the aqueous phase. After centrifugation, the orange SUP-AZO liquid droplet at the bottom of the vial was separated from the
aqueous supernatant. The supernatant was removed by a pipette and the SUP-AZO liquid material was collected for further characterization.

**Characterization of the SUP-AZO complex fluids**

Polarized optical microscopy (POM) was conducted on a Zeiss Axiophot. Thermogravimetric analysis (TGA) was carried out using a TA Instruments Q1000 system in a nitrogen atmosphere and with a heating/cooling rate of 10°C/min. Small-angle X-ray scattering (SAXS) was performed by employing a conventional X-ray source with radiation wavelength of $\lambda = 1.54$ Å and a Bruker Nano/microstar machine was used to obtain small angle scattering profiles, where the sample-to-detector distance was 24 cm. The sample holder is a metal plate with a small hole (diameter ~0.25 cm, thickness ~0.15 cm), where the X-ray beam passes through. The SUP-AZO liquid sample was loaded into the hole by a pipette and was then sealed by kapton. The scattering vector $q$ is defined as $q = 4\pi \cdot \sin(\theta)/\lambda$ with $\theta$ being the scattering angle. A shear strain controlled Bohlin VOR rheometer (Bohlin Reologi AB) with two stainless steel fixtures was used to study shear-induced disorder-order phase transition of the SUP-AZO fluid materials.
Supplementary figures

Figure S8. Representative photographs showing the preparation of a SUP-AZO fluid exemplified for E144-AZO. (A) Upon mixing aqueous solutions of SUPs and AZO surfactant, the yellow solution became cloudy. (B, C) After centrifugation, the SUP-AZO complex with orange color was separated from the aqueous supernatant by a pipette. (D, E) Photographs showing the gravity-induced flow behavior of the SUP-AZO liquid droplet at room temperature.
Figure S9. Stoichiometry analysis of the E18-AZO complex by \(^1\)H-NMR (400 MHz). NMR information was recorded when the lyophilized E18-AZO complex was dissolved in deuterated DMSO. The signals of terminal methyl group (marked by a) in AZO and methyl group of Valine (marked by c) in SUP were utilized to estimate the molecular ratio of SUP and AZO. The proton in aromatic region (marked by b) was used as an internal standard. The binding stoichiometry can be calculated by the integration of methyl protons difference between the AZO and E18-AZO complex. Assuming that one SUP molecule could combine with \(n\) × AZO molecules (SUP:nAZO), then after complexation, the total number of methyl protons can be shown as: \((\text{SUP}(V_{22})) \times 6 + (\text{AZO}(-\text{CH}_3)) \times n\). According to the integration of the methyl protons of AZO and E18-AZO in their \(^1\)H-NMR as shown above, we will have:

\[
22 \times 6 + 3.04 \times n = 8.28 \times n
\]

\(n=25\)

so the stoichiometric ratio of AZO and E18 is 25.
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Figure S10. Thermogravimetric analysis (TGA) of the SUP-AZO fluids. TGA analysis showed that the SUP-AZO fluids exhibited water contents of 50-60% (w/w) (black curve, E72-AZO; red curve, E144-AZO).

Figure S11. SAXS pattern of the shear-induced phase transition of the SUP-AZO fluids shown for E144-AZO. Capton was used to seal the SUP-AZO samples and appears in the pattern. SAXS was measured after cessation of shear. The broad diffraction ring at 1.5 nm$^{-1}$ indicated the formation of nematic phase by mechanical shearing.
Chapter 2

Figure S12. SAXS measurement of a SUP-AZO liquid droplet representing a non-treated and non-aligned sample. (A, B) 10 μL of the freshly prepared E144-AZO liquid was transferred to a metal plate, where a small hole (diameter ~0.25 cm, thickness ~0.15 cm) was used to host the bulk droplet and two kapton plates were utilized to seal the small hole to avoid water evaporation of the liquid sample. X-ray beam (diameter ~0.5mm) passes through the small hole to measure the droplet. (C) SAXS pattern of the E144-AZO liquid droplet. The absence of any diffraction pattern from the E144-AZO sample suggests that it is an isotropic liquid.
Figure S13. Stability of the shear-induced nematic LC phase of the E144-AZO fluid over time. (A-D) After cessation of shear, the birefringence textures were preserved over time in the shear-induced nematic LC phase. Scale bar is 100μm. (E, F) SAXS results of the shear-induced E144-AZO liquid, which were recorded after 4 hours after cessation of shear. The data suggests that the long-range ordered nematic LC phase is stable over time period investigated. Note, the complete sealing of the liquid system is hard over longer periods of time and slow water evaporation took place. After longer observation times (> 9 hours), a smectic phase was formed due to lower water content, which is discussed in the following Figure S15.
Figure S14. POM investigation of the dependence of temperature on the phase transition of the E144-AZO complex (here 1 μL of the E144-AZO liquid was applied between two glass substrates). The shear-induced nematic phase disappeared above 125°C. To avoid large degree of water evaporation, a high heating rate (50°C/min) was applied. Scale bar is 100 μm.
Figure S15. Structure evolution of the shear-induced nematic SUP-AZO fluid system upon water evaporation. When the water content of the E144-AZO complex drops below 25% (A, D), a large birefringent domain with uniform molecule orientation was observed (B). The 1st and 2nd order diffraction patterns indicate a long-range ordered smectic phase of the E144-AZO complex with preferential layer alignment (C, E, F). Based on rough estimation of volumes and comparison with the experimental data, when the SUP-AZO system contains water content of ~14%, the layer spacing of 8.9 nm suggests the lamellar structure is composed of a hydrated SUP sublayer ~1.3 nm thick that electrostatically interacts with two sublayers of AZO/H2O of ~7.6 nm. These results suggest that the nematic SUP-AZO can be transformed into a smectic phase by water evaporation.
Figure S16. Investigation of the relationship between charge density of SUPs and the shear-induced phase transition of the SUP-AZO fluids involving E18-AZO and EE36-AZO. (A, B) POM analysis of the E18-AZO liquid before and after application of shear force. No nematic phase was observed even after shearing. (C, D) POM analysis of the EE36-AZO liquid before and after application of shear force. After exertion of shear, a nematic LC phase was formed. The right images were captured after cessation of shear. Scale bar is 100μm.

Figure S17. POM analysis of shear-induced phase transition of EE108-AZO fluid. Nematic LC phase was formed after shearing. The right image was captured after cessation of shear. Scale bar is 100μm.
Figure S18. Investigation of the dependence of molecular weight of the SUPs on the shear-induced phase transition of the SUP-AZO fluids including E18-AZO, E36-AZO, and E72-AZO. (A, B) POM analysis of the E18-AZO liquid before and after the application of shear force. No nematic phase was induced even after shearing. (C, D) POM analysis of the E36-AZO liquid before and after the application of shear force. Nematic LC phase was formed upon a mechanical stimulus. The right images were captured after cessation of shear. Scale bar is 100μm.
Figure S19. POM analysis of the E36-AZO fluid samples after applying shear force at a fixed frequency (1Hz) and strain (4.9). The resulting material exhibited very weak LC birefringence after 13 minutes. The right image was captured after cessation of shear. Scale bar is 100μm. Note, the weak “background” birefringence in A and B were originated from the used plastic films.

Figure S20. POM analysis of the E144-AZO fluid material after the application of water flow at a flow rate of 20 mL/sec (5 minutes water flushing). At this flow rate, no ordered LC phase was induced. Note, the weak “background” birefringence was originated from the used plastic film.
Reference


