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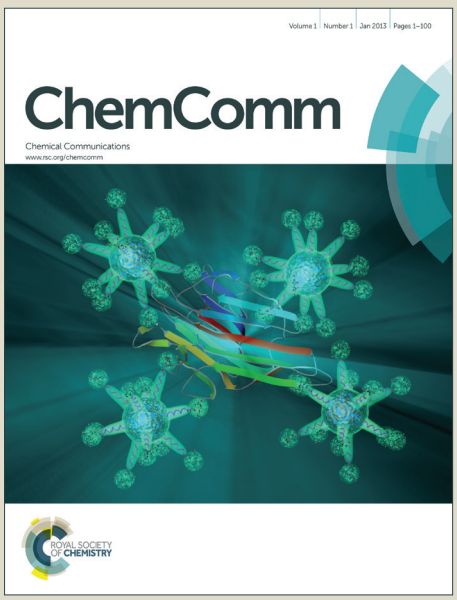

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Oligopeptide-decorated Liquid Crystal Droplets for Detecting Proteases

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We prepared the bioactive liquid crystal (LC) droplets by decorating oligopeptide at the LC/aqueous interface to stabilize the LC droplets. These LC droplets can be used to detect proteases through the specific cleavage of protease on oligopeptide and lead a bipolar-to-radial transition of LC configuration inside the droplets.

Liquid crystal (LC) droplets have been considered as a more promising platform among LC-based sensing applications.[1-4] Comparing to their LC/solid[5] and LC/aqueous[6] counterparts, LC droplets exhibit large surface-area-to-volume ratio which facilitates the reorientation of LCs at the interface. In addition, LC droplets possess more director configurations, which enable them to provide more in-depth quantitative information of analytes.[7-13] For the stability of LC droplets as a sensing platform, amphiphilic molecules are required to adsorb at the LC/aqueous interface to against self-coalescence in the solution. For example, Yang and coworkers utilized polyethylene imine (PEI) as amphiphiles to prepare LC droplets, followed by the immobilization of IgG on the surface for detecting anti-IgG.[14] However, the preparation of these LC droplets requires layer-by-layer assembling of functional molecules on the surface of droplets, resulting in the batch variations of the properties of LC droplets. In addition to stabilizing LC droplets, amphiphiles such as biological lipids can also act as the probe in a sensing system such that the preparation of LC droplets is simplified. For example, Abbott and coworkers developed the phospholipid L-dipalmitoyl phosphattidylcholine (L-DLPC)-decorated LC droplets as a protease assay for detecting phospholipase A_2.[15] The preparation of droplets was simply achieved by equilibrating the naked LC droplets in the solution of L-DLPC vesicles. Nevertheless, this approach was suffered from the specificity issue and only applicable to limited number of targets.

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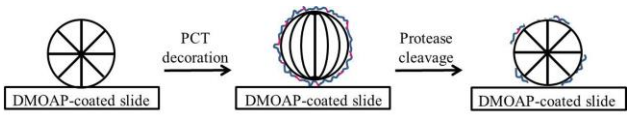
Oligopeptides have been widely used as recognition molecules in many sensing platforms to detect various targets such as proteases,[16, 17] proteins,[18, 19] neonicotinoids,[20] and metal ions.[21, 22] The advantage of using oligopeptides as the recognition molecules is that it provides a significant improvement to the target specificity. Thanks to the well-developed protocol for peptide synthesis, the sequence of oligopeptide can be custom-made which opens the variety of choosing targets. By introducing both hydrophobic and hydrophilic residues into the sequence of an oligopeptide, we feel that oligopeptide can also act as amphiphiles to decorate at the LC/aqueous interface to stabilize the LC droplets in aqueous solution.

To proof this concept, we selected the oligopeptide (PCT: CKK-KYGSNYTYIDEGNQYATYGGGGYETSAAYGYKKKC) as the amphiphile for the preparation of LC droplets. Among the 38 amino acid residues of PCT, 17 amino acid residues are hydrophilic (K, S, N, T, D, E and Q) while 14 amino acid residues are hydrophobic (C, Y, I and A). The protocol for preparing the LC droplets in this report is very straightforward. We simply mixed nematic 5CB with the solution containing PCT and glutaraldehyde (as a cross linker to form covalent bonds with the amine groups at N-terminal or on the lysine residues) under reduced condition, followed by shearing the mixture using a vortex mixer for 15 min to give the desired droplets. The droplets were then moved onto a DMOAP-coated glass slide and the optical textures of LC droplets were studied under crossed polars. To study how oligopeptide affects the orientation of the LC inside droplets, we carried out series of experiments to observe the optical texture of LC droplets prepared by different concentrations of PCT in the mixture. It was found that when the concentration of PCT is 0, 1 and 10 μg/mL, LC droplets show a radial configuration characterized by a single point of defects at the center of the droplets. Whereas when the concentration of PCT is 100 and 1000 μg/mL, LC droplets show a bipolar configuration characterized by two points of defects at the edge of the droplets (Fig. 1). Such concentration-dependent configuration of LC droplets suggests that PCT adsorbed to the LC/aqueous interface and altered the anchoring energy of LCs,



Scheme 1 Schematic illustration for the transition of the configuration of LC droplets by PCT decoration and protease cleavage.

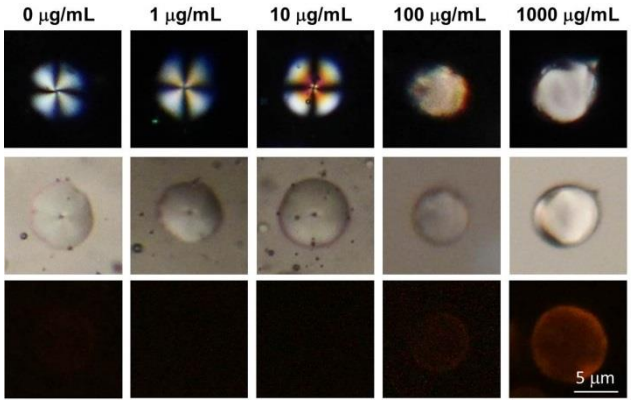


Fig. 1 Polarized (top row), bright field (middle row) and fluorescence (bottom row) images of the LC droplets prepared by different concentrations of PCT when they are deposited on DMOAP-coated slide. For the fluorescence images, Cy3-labelled PCT was used to prepare LC droplets.

which triggered the radial-to-bipolar ordering transition of LCs inside the droplets (a so-called adsorbate-induced anchoring transition).[10] Based on our findings, the minimum concentration of PCT to induce the transition of LC is 100 μg/mL. This value is close to the critical micelle concentration of PCT (203 μg/mL) estimated by using pyrene as a fluorescence probe (Fig. S1). Unlike the previous study which demonstrated that the configuration of LC droplets is size-dependent and the LC droplets with the diameter lager than 3 μm showed bipolar configuration,[15] our results showed that the configuration of LC droplets (without the decoration) is radial even when LC droplets are larger 3 μm. This corresponds to the deposition of droplets on the DMOAP-coated slide, which induces the transition of LC inside the droplets from bipolar to radial configuration. This phenomenon was also observed in recent studies showing that the LC droplets without any surface modification displayed radial configuration when supported on OTS-coated surface.[8, 9] However, when PCT was decorated on the surface of LC droplets, PCT hinders the approaching of LCs to the DMOAP-coated slide and induces another transition of LC configuration from radial to bipolar inside the droplets (Scheme 1). According to past studies, the hydrophobic and/or π–π interactions between the phenyl groups of 5CB and the amphiphile at the interface lead to a bipolar configuration of LC droplets.[23] Therefore, the bipolar configuration of PCT decorated LC droplets may be attributed to the 8 tyrosine (Tyr, Y) residues, which has a phenol moiety on its side chains, on PCT. To further investigate on this point, we designed another oligopeptide (PT: CKKKRGSNRTRIDEGNQRATRGGGGRETSA-ARGRKKKC) of which the sequence is very similar to PCT. The only difference is that we replaced all tyrosine residues into arginine (Arg, R) residues. The LC droplets prepared by 1000 μg/mL of PT show radial configuration on DMOAP-coated slide (Fig. S2); however, it was found that most of droplets in the solution aggregated after 10 min. This phenomenon can be explained by the failure adsorption of PT to LC droplets, which further implies that the interactions between tyrosine and 5CB are crucial to the adsorption of oligopeptide on LC droplets and the formation of bipolar configuration. To further characterize the decoration of PCT on LC droplets, we prepared the LC droplets by using the oligopeptide which is pre-labeled with a fluorescence tag (Cy3-PCT). The images in the bottom row of Fig. 1 show that the droplets prepared by 1000 μg/mL of Cy3-PCT are fluorescent, especially at the margin of the droplets. This result suggests that the decoration of PCT is located at the surface of the droplets. In addition, we also noticed that the fluorescence intensity decreases as the concentration of Cy3-PCT decreases. When the concentration of Cy3-PCT is lower than 10 μg/mL, no fluorescence can be observed. This phenomenon is in accordance with the result from polarized images showing that the decoration of PCT on LC droplets is indeed concentration-dependent. Higher concentration of PCT results in higher surface density of PCT on the LC droplets. Furthermore, the ζ-potential of the LC droplets prepared by 1000 μg/mL of PCT was measured to be -21.6 mV at pH 9.3 and -5.4 mV at pH 7.6. Previous study has reported that the ζ-potential of bare 5CB droplets is around -65 mV and -50 mV in water at pH 8.6 and 7.0, respectively.[24] Besides, the isoelectric point PCT is 9.09,[25] so PCT should be nearly neutral at pH 9.3 while positively charged at pH 7.6. Therefore, the significant increase in ζ-potential of LC droplets evidenced the decoration of PCT on the surface of LC droplets. This result also implies that the surface of LC droplets are not fully covered by PCT as the ζ-potential of the droplets is not solely characterized by PCT. All the results above lead us to conclude that oligopeptide can be decorated on the surface of LC droplets and affects the LC configuration inside the droplets. From the preparation perspective, we want to highlight the idea of using oligopeptide as amphiphiles to prepare LC droplets because it would not only stabilizes the LC droplets but also creates their bioactivity.

Because the stability of LC droplets is an important issue to be concerned as a sensing platform, our next goal is to study the effect of oligopeptide concentration on the stability of LC droplets. This can be done by statistical analysis of LC droplets in terms of their size distribution under microscope. Fig. 2 shows that when there is no PCT decorated on the droplets (the concentration of PCT is 0 μg/mL), the LC droplets are polydisperse with the diameter ranging from 14 to 30 μm in 10 min. After 30 min, however, no LC droplets were observed under cross polars and we found that the LCs and the aqueous solution have been separated in to two individual layers. This result implies that LC droplets without PCT-decoration coalesce in aqueous solution very easily. When LC droplets were decorated by PCT, LC droplets also coalesced in 10 min when the concentration of PCT is 1 and 10 μg/mL; however, they were able to withstand coalescence for 3 days without changing their configuration when the concentration of PCT is 100 μg/mL. Surprisingly, LC droplets prepared by 1000 μg/mL of PCT can withstand coalescence without changing their configuration for 30

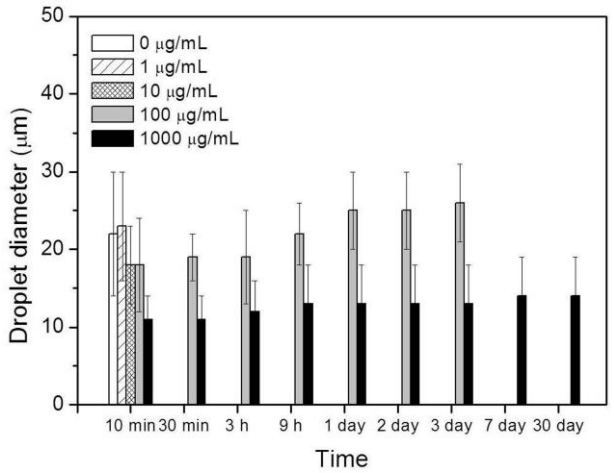


Fig. 2 Histogram of the diameter of LC droplets prepared by different concentration of PCT in PBS buffer.

days. These results suggest that the stability of LC droplets could be greatly enhanced through the decoration of PCT. Previous study reported that the stability of LC droplets is determined by the nature of molecular interactions between the amphiphile and LC. Based on our observations, the stability of PCT-decorated LC droplets is comparable with the polyvinyl alcohol (PVA)-decorated LC droplets (also stable for more than 30 days), which have strong hydrogen bonding between PVA and LC molecules.[26] If we look into the size distribution of the droplets, it was found that when the concentration of PCT is 100 and 1000 μg/mL, the diameter of the droplets is ranging from 12 to 24 μm and 8 to 14 μm in 10 min and increases 44% and 18% after 3 days, respectively. This means the effect of PCT stabilizing LC droplets is concentration-dependent. Furthermore, we also found that the cross linker is responsible to the stability of the droplets. Without using glutaraldehyde as cross linker, all LC droplets coalesced after 1 h, which means the decoration by PCT along is not capable of resisting LC droplets from coalescence. A possible explanation to this phenomenon is that glutaraldehyde formed covalent bonds with amine groups at N-terminal or on the lysine residues of the oligopeptide, which allowed PCT to form larger cross-linking network on the surface of LC droplets to encapsulate the droplets from coalescing with each other.

To test whether the PCT-decorated LC droplets can be applied as a sensing platform for proteases, we immersed PCT-decorated LC droplets in the solution containing 5 μg/mL of α-chymotrypsin on a DMOAP-coated slide. To pursue the stability of droplets, we selected the LC-droplets prepared by 1000 μg/mL of PCT as the substrate of protease cleavage. Fig. 3a shows that all LC droplets exhibit radial configuration after exposing to α-chymotrypsin for 1 h, which means α-chymotrypsin triggers a bipolar-to-radial transition of the PCT-decorated LC droplets. Because α-chymotrypsin cleaves the amide bond of Y residues and there are 8 Y residues on PCT, the transition of the LC configuration can be explained by that α-chymotrypsin cleaves the PCT into many shorter fragments such that breaks the rigidity of oligopeptide network on the surface of droplets. When the fragments desorbed form the surface, the configuration of LC droplets is directed by the DMOAP-coated slide so radial configuration was observed (Scheme 1). This result is consistent with the fact that the droplets with lower surface density of PCT exhibit radial configuration of LCs when they are deposited on DMOAP-coated slides. By comparing the fluorescence intensity of LC droplets decorated by Cy3-labeled PCT before and after they were immersed in in the solution containing 5 μg/mL of α-chymotrypsin for 1 h, we could estimate that 55% of oligopeptide fragments were released from the LC surface after cleavage (Fig. S3). To determine the limit of detection (LOD) of this system, we employed series of experiments to observe the optical response of the LC droplets in the solution containing different concentrations of α-chymotrypsin. It was found that LC droplets show radial configuration only when the concentration of α-chymotrypsin is 5 μg/mL (Fig. 3a). In contrast, LC droplets show both radial and bipolar configuration when the concentration of α-chymotrypsin is 0.5 μg/mL (Fig. 3b), while they show bipolar configuration only when the concentration of α-chymotrypsin is lower than 0.05 μg/mL (Fig. 3c and 3d). As a result, the LOD for α-chymotrypsin by using PCT-decorated LC droplets as a sensing platform is approximately 0.5 μg/mL. To test the specificity of this system, we also mixed PCT-decorated LC droplets with the solutions containing 5 μg/mL of trypsin and 5 μg/mL of CNBr, respectively. Trypsin cleaves peptide bonds at the C-terminus of arginine (Arg, R) and lysine (Lys, K), while CNBr cleaves peptide bonds at the C-terminus of methionine (Met, M). It was found that all LC droplets show radial configuration in the solution containing trypsin (Fig. 3e), which means trypsin is also able to trigger the transition of LCs inside the PCT-decorated droplets by cleaving the 6 lysine (Lys, K) residues on PCT. In contrast, all LC droplets show bipolar configuration in the solution containing CNBr (Fig. 3f), which means CNBr cannot trigger the transition of LCs inside the PCT-decorated droplets. This can be explained by that there is no methionine residue on PCT to be cleaved by CNBr. To examine whether α-chymotrypsin itself can induce the transition of LC droplets without the decoration of oligopeptide on LC droplets, we compared the results when bare LC droplets was mixed with α-chymotrypsin and PBS buffer. We found the configuration of LC droplets is radial no matter α-chymotrypsin is present or not (Fig. 3g and 3h), which means α-chymotrypsin cannot induce bipolar-to-radial transition if LC droplets are not

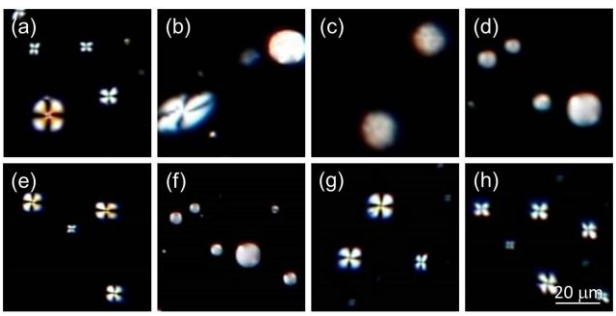


Fig. 3 Polarized images of PCT-decorated LC droplets in (a) 5 μg/mL α-chymotrypsin, (b) 0.5 μg/mL α-chymotrypsin, (c) 0.05 μg/mL α-chymotrypsin, (d) PBS buffer, (e) 5 μg/mL trypsin and (f) 5 μg/mL CNBr after 1 h. (g) and (h) show the polarized images of bare LC droplets in 5 μg/mL α-chymotrypsin and PBS buffer, respectively.

decorated with PCT. Furthermore, we carried out HPLC experiments to study whether PCT decorated on the LC droplets was cleaved by proteases. It was found that the chromatogram of PCT solution shows only one peak attributed to the uncleaved PCT. In contrast, after PCT-decorated LC droplets were mixed with 5 μg/mL trypsin solution for 1 h, the total number of peak increases to twelve, which means PCT was cleaved and the fragments were released into the aqueous solution (Fig. S4). All these results, when combined, lead us to conclude that oligopeptide decorated on the surface of LC droplets could be selectively cleaved by proteases present in the aqueous solution, which demonstrates the great potential of such LC droplets to be utilized as the biological sensing platforms.

In conclusions, we have successfully prepared the oligopeptide decorated LC droplets with the interesting bioactivity. These LC droplets proved to be stable as they could withstand coalescence without altering their configuration for 30 days. We have also found that the LC droplets decorated with the oligopeptide with 8 tyrosine residues showed bipolar configuration. The specific cleavage of α-chymotrypsin on the oligopeptide leads to desorption of oligopeptide fragments from the surface and resulted in a bipolar-to-radial transition of the LC configuration. By using this platform, serine proteases such as α-chymotrypsin and trypsin can be detected, and it did not respond to CNBr and pure PBS buffer solution. Because the transition of LC configuration inside the droplets is size-dependent, the performance of this platform could be optimized if the size and number of LC droplets are precisely controlled. Some previous studies have demonstrated that monodisperse LC droplets with various diameters can be prepared by using polymeric shells[7] or microfluidics.[12] Besides, it has also been reported that monodisperse LC droplets can be patterned on a solid surface with desired number and size using ink-jet printing.[27] Therefore, we believe that the concept of using oligopeptide as amphiphiles to decorate LC droplets provides a unique opportunity for developing a highly sensitive detection system for various targets just by changing the sequence of oligopeptide decorated on LC droplets. The fabrication of LC droplets is straightforward and cost-effective. The read-out of this system does not require sophisticated instrumentation, and the result can be easily interpreted by the untrained users. These advantages show the potential of LC droplets in commercial applications.

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