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# Brownian Motion Retarded Polymer-Encapsulated Liquid Crystal Droplets Anchored over Patterned Substrate via Click Chemistry†

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Highly sensitive liquid crystalline (LC) materials are promising candidates for sensing applications. Anchoring of liquid crystal (LC) droplets over substrates can reduce the signal broadening due to retarded Brownian motion. In addition, anchoring over patterned substrates can facilitate the precise quantification of spatially resolved detection of analyte. To this end, we report a versatile approach to anchor LC droplets encapsulated within polymer capsules over patterned substrate via highly stable triazole linkage created through click chemistry. Confocal and polarized microcopy images confirm the anchoring of LC droplets over the glass substrates. Change in orientation (bipolar to radial) due to the binding of sodium dodecyl sulfate (SDS) surfactant and 1,2-dilauroyl-sn-glycero-3-phosphocholine ($_L$-DLPC) at the LC interface suggests that the sensing capabilities of the Brownian motion-retarded encapsulated LC droplets is retained. It is also demonstrated that the so assembled systems are stable over six months, which makes them potential candidates for portable microfluidic sensors.

## Introduction

Liquid crystalline materials are promising candidates for fabricating cost-effective and portable bio/chemical sensors, interfacial and optoelectronic applications.[1-4] Orientation of liquid crystal (LC) is highly sensitive to molecular events such as interfacial adsorption of chemical and/or biological molecules.[1-5] In order to explore the behavior of aqueous/LC interface, past investigations have largely focused on the preparation of LCs thin films and LC droplets emulsions. LC thin films at pretreated substrates possess the main advantages of high sensitivity, easy fabrication and handling of portable devices (e.g. microfluidic chips, bio/chemical sensors, etc). Pretreated substrates drives the LC to orient in the "bipolar" mode, which in the presence of phospholipids,[1, 4] bacteria,[4, 6] viruses,[7-9] heavy metals,[7, 10] DNAs,[11] and surfactants[11-14] is modified to "radial" mode, facilitating the detection of the signal. The sensitivity of the signal change is dependent on the interfacial area available for binding events of the analyte. A 2-D geometry imposed by the thin film geometry decreases this interfacial area as only one side is available for binding, with the other side already utilized for binding to the pre-treated substrates. In contrast to the 2-D geometry, higher surface area 3-D spherical geometry is preferred for enhanced sensitivity and this is made available if LC is deployed in the form of droplets. This feature has been validated in recent studies.[15] Additionally, the LC orientation transition is sensitive to the size of LC droplets and in contrast to LC thin film geometry, droplet geometry does not need any surface treatment to define the LC orientation. However, such spherical droplets are susceptible to Brownian motion which in turn results in spatial averaging of the signal, thereby decreasing the spatial resolution of the signal.[16-18]

Firstly, to reduce the signal broadening due to Brownian motion and to prolong the observation of binding event with single LC droplets, it is effective to anchor the LC droplet over planar substrates. Further, the anchoring strategy should not modify the sensing/inherent properties of the liquid crystal. An attempt has been made by Abbott and his co-workers to immobilize the polymer-decorated LC droplets on substrate through electrostatic/covalent interactions. While electrostatically immobilized polymer decorated LC droplets possess limited stability, immobilization via covalent interaction does not increase the stability beyond 10 minutes because of the complete hydrolysis of azlactone functionality in the aqueous systems.[15] Hence, increasing the stability of anchored LC droplet on planar solid substrate, with minimal modifications of LC properties, is critical for utilizing them in sensing applications.

Secondly, an issue that is of practical importance in the fabrication of microfluidic sensors is to spatially resolve the concentration of the analyte.[19-22] This is typically done by differentially patterning the sensing element for precise spatial and temporal detection of analyte concentration and detection of single molecules for extended period of time.[19-23]

To address the above mentioned two issues, we demonstrate an approach towards fabricating highly stable anchored LC droplets encapsulated polymer capsule over patterned surfaces. The anchoring is achieved by forming strong triazole linkages between alkyne/azide functionalized polymer capsules on azide/alkyne functionalized planar solid substrates via click chemistry. This approach can be generalized in multiple ways. In a manner similar to the physical encapsulation within the

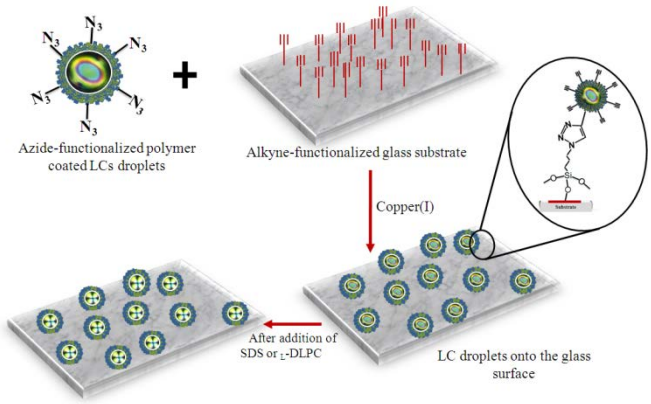
polymer capsules, a variety of other LC droplet/other hydrophobic liquids can be encapsulated without affecting their inherent properties. This also offers an approach towards studying the kinetics of several reactions (e.g. Ligand-substrate binding) in the micro and nano scale range. As it has been shown in our earlier work, [24] the anchoring of polymer capsules can be generalized towards a variety of substrates (such as glass, quartz, silicon, ITO, stainless steel etc.) and hence provides considerable flexibility in the fabrication of devices. This highly scalable approach results in the generation of highly stable triazole linkages for anchoring encapsulated LC droplets to the substrates, thereby, restricting the Brownian mobility of the LC droplets.

In this communication, we studied the ordering transition of anchored nematic LC droplets (4-cyano-4′-pentylbiphenyl) over patterned glass substrates via click chemistry in the presence of sodium dodecyl sulfate (SDS) surfactant and 1,2-dilauroyl-sn-glycero-3-phosphocholine($_L$-DLPC) for biosensing application. We observed that these patterned LC droplets were highly stable over six months, which we attribute to the strong triazole linkage formed between substrate and LC droplets via click chemistry. These results suggest that the proposed approach pave the pathway for quantitative analysis with individual LC droplets for variety of applications by restricting the Brownian mobility of LC droplets.

For anchoring of LC droplets on planar substrate, we adapt a strategy that LC droplets are encapsulated in a hollow polyelectrolyte multilayer capsule (prepared by layer-by-layer approach). The final poly (acrylic acid) polymer layer of the capsule possess the click moieties (alkyne/azide) which facilitates the anchoring on to the planar substrate possessing the complementary click moieties (azide/alkyne). LC droplets encapsulated polymer capsule have been prepared by adapting the procedure from the earlier report, in which LC droplets were infiltrated within the hollow polymer capsules prepared through a template based approach.[25] Polymer capsules were prepared by cyclical deposition of negatively charged polystyrene sulfonate (PSS) followed by positively charged polyallylamine hydrochloride (PAH) polymers on colloidal positively charged silica particles, with the cycle being repeated four times.[25] The eighth layer of PAH polymer was fluorescently labeled with Rhodamine isothiocyanate (RITC) dye to assist the characterization by confocal laser scanning (CLSM). Further, an additional layer (ninth layer) of alkyne/azide functionalized poly (acrylic acid) ($PAA_{Alk}$/$PAA_{Az}$) polymer was assembled on PAH-RITC layer to facilitate the click reaction (see supporting information). Subsequently, the silica core was removed through etching with hydrofluoric acid and this was followed by LCs infiltration. Depending on whether the polymer is functionalized with azide or alkyne group, the glass substrate surface is functionalized by the complementary alkyne or azide group respectively so as to form the triazole linkage (see ESI†).

Presence of alkyne and/or azide click moieties on polymer and glass substrate was confirmed through $^1$H NMR and FTIR respectively (see ESI†). The FTIR spectra of azide and alkyne functionalized glass substrate (see Fig. S1 and S2†) show peaks at 2250 cm$^{-1}$ and 2270 cm$^{-1}$ which confirms the presence of azide and alkyne groups on glass substrates. Presence of azide and alkyne group on poly (acrylic acid) polymer was confirmed through $^1$H NMR peaks at 3.66 and 1.95 ppm (see Fig. S3 and S4†).[26]

Scheme 1 shows a schematic representation for anchoring azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized planar glass substrate via click chemistry.




**Scheme 1** Schematic representation for anchoring azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized glass substrate via click chemistry.

Alkyne-functionalized glass substrate were immersed in an aqueous colloidal solution of azide-functionalized LC droplets encapsulated polymer capsule followed by addition of copper sulfate (10 mM) and ascorbic acid (50 mM) aqueous solution in 1:2 ratio to catalyze the click reaction for 12 h. Further, the substrates were washed thrice with water to remove any physically adsorbed LC droplets and later preserved in aqueous solution. On the other hand, scheme S1† shows a schematic representation for anchoring of alkyne-functionalized LC droplets encapsulated polymer capsule on azide-functionalized planar glass substrate via click chemistry.

Formation of polymer capsules and encapsulation of LC droplets inside the polymer capsules were confirmed through CLSM and polarized light microscopy (PLM) (see Fig. S5†). Fig. S5a and S5b† show the CLSM images of hollow polymer capsule and LC droplets encapsulated polymer capsule in aqueous solution respectively where red emission confirms the presence of RITC-labeled polyallylamine hydrochloride eighth layer. PLM images (see Fig. S5c†) shows the bipolar orientation (i.e. two point defects at the poles of LC droplets) of LC droplets encapsulated polymer capsule aqueous solution which matches with previous reports.[27] Fig. 1a and b show bright-field microscopy (BFM) and PLM images of anchored azide-functionalized LC droplets encapsulated polymer capsule on alkyne functionalized glass substrate via click reaction respectively, whereas Fig. 1c shows corresponding fluorescence micrograph. It is noted that the displacement of the LC droplets encapsulated polymer capsules is minimal, which suggests the retardation of Brownian motion of LC droplets. Further, contrast in the bright field images clearly suggests that the anchored LC droplets were encapsulated in polymer capsule. Polydispersity in polymer capsules (Fig.1) is due to the polydispersity in silica template. Furthermore, PLM images reveals that anchored LC droplets have bipolar orientation (and bipolar defects indicated by white arrow in BFM image of Fig. 1a) which is same as free LC droplets. This clearly suggests that the interface between the LC droplets and water is unaffected during the click reaction which further proves that this approach does not modify the sensing/inherent property of LC droplets.

Further, fluorescence microscopy images (Fig. 1c) of anchored LC droplets encapsulated polymer capsule clearly suggest that the integrity of the polymer capsules are maintained after anchoring over the glass substrate via click chemistry.

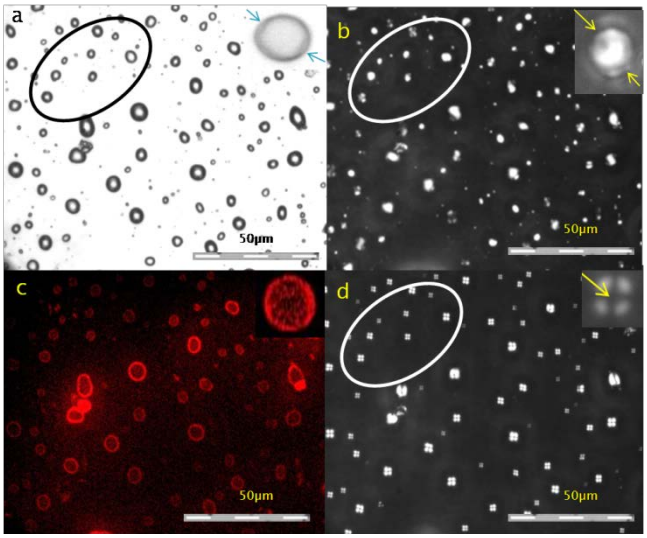



**Fig. 1** (a) Bright field, (b) polarized light and (c) fluorescence microscopy images of anchored azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized glass substrate via click chemistry and (d) polarized after the addition of SDS. Scale 50µm.

In order to investigate the sensing capability of the anchored liquid crystal droplets in the presence of biomolecules, we have studied their interaction in the presence of SDS surfactant and $_L$-DLPC lipid as a model biomimetic adsorbate. A PLM image (Fig. 1d) of anchored LC droplets after addition of SDS surfactant clearly shows the change in the orientation of LC droplets from bipolar to radial orientation. This ordering transition in LC is a result of changes in interfacial energy of the LC interface following the adsorption of analyte. As LC droplets can show reorientation solely due to confinement within a polymer capsules < 3 um (i.e. even in the absence of analyte),[28] which makes it difficult to deconvolute the orientation changes due to decrease in size of LC and due to adsorption of the analyte. In order to avoid this difficulty, we have restricted the silica enclosure to being > 3 µm.

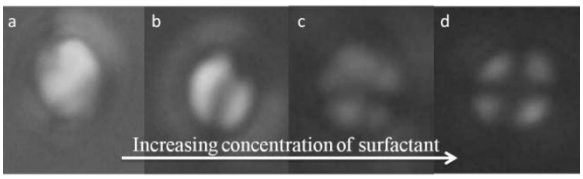



**Fig. 2** Polarized light images of anchored azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized glass substrate via click chemistry after addition of SDS solutions of 0.2mg/ml, 0.5mg/ml, 1mg/ml and 6mg/ml concentration. Scale 5µm.

Encircled portion in Fig. 1a, b and d shows that the location and the number of LC droplets were unchanged with the addition of surfactant. This further confirms that LC droplets encapsulated polymer capsule were firmly anchored on to the glass substrate and their Brownian motion has been retarded.

On the other hand, we observed greater Brownian motion in unanchored LC droplets encapsulated polymer capsule over glass substrate. Further, we observed that there is negligible change in the orientation/location/number of anchored LC droplets over the glass substrate over six months in aqueous solution (see Fig. S6†). We attribute this high stability to triazole linkages that anchors LC encapsulated polymer capsules to glass substrates, making them a potential candidate for fabrication of portable biosensors.

Further, to illustrate the advantage of anchoring, we performed a quantitative study focused on an individual LC droplet. The differential response of the individual droplet towards different concentration of SDS surfactant (i.e. 0.2mg/ml, 0.5mg/ml, 1mg/ml and 6mg/ml) was observed. PLM (Fig. 2a-d) shows the gradual movement of two-point defects from the poles of LC droplet to the centre of LC droplets for SDS concentration more than 1mg/ml which results in the change of LC droplets orientation from bipolar to fully radial through intermediate transition steps.[27, 28]

In general, the sensitivity of the sensor can be characterized by two factors: 1) Response time 2) Minimum concentration of the analyte that can be detected by the sensor. We anticipate that the response time will decrease with decrease in the number of polymer bilayers. This issue has been addressed in the literature where it was shown that introduction of polymer bilayers decreases the response time.[27] However, increase in the thickness of the bilayer increases the stability of the polymer capsules. Hence, the trade-off is between decreasing response time vs. increasing stability. The minimum concentration of the analyte that can be detected by the sensor depends on specific analyte to be detected and the LC utilized for detecting the analyte. The approach demonstrated in the present study is general enough in the sense that it can be extended to any LC that can be infiltrated via the polymer capsule.

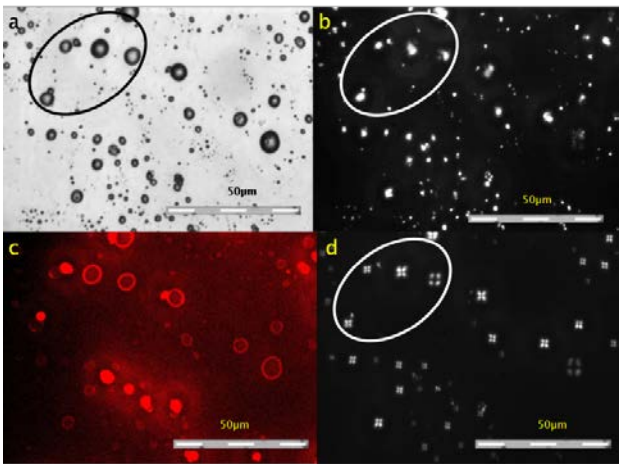



**Fig. 3** (a) Bright field, (b) polarized light and (c) fluorescence microscopy images of anchored azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized glass substrate via click chemistry and (d) polarized after the addition of $_L$-DLPC. Scale 50µm.

Hence, if one has to increase the detection limits, an approach towards that goal will be to search for more sensitive LC systems that can be infiltrated via the polymer capsule.

Additionally, we have also studied the effect of $_L$-DLPC phospholipids on LC droplets orientation to investigate label-free detection of molecular species in aqueous stream. BFM, PLM and fluorescence microscopy images of anchored azide-functionalized LC droplets encapsulated polymer capsule on alkyne-functionalized glass substrates via click reaction are shown in Fig. 3a-c. Polarized light image shown in Fig. 3d clearly reveals the transition of LCs orientation from bipolar (Fig. 3b) to radial orientation after addition of $_L$-DLPC. Further, encircled portion shown in Fig. 3a, b and d confirms that location and number of LC droplets encapsulated polymer capsule were unchanged after the addition of lipid due to retarded Brownian motion.

In order to demonstrate the versatility of proposed approach, we have also anchored the alkyne-functionalized LC droplets encapsulated polymer capsule on azide-functionalized planar glass substrate via click reaction (Scheme S1†). This is important especially in certain systems/substrates wherein one of the interfaces is more amenable for functionalization either via azide or via alkyne only.[24]

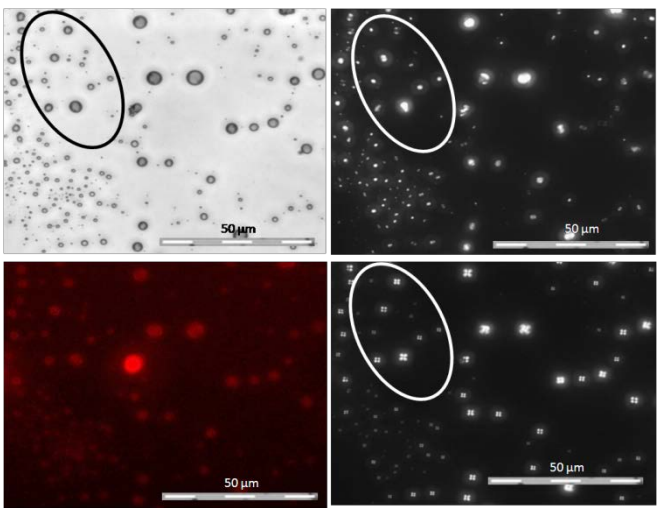



**Fig. 4** (a) Bright field, (b) polarized light and (c) fluorescence microscopy images of anchored alkyne-functionalized LC droplets encapsulated polymer capsule on azide-functionalized glass substrate via click chemistry and (d) polarized after the addition of SDS. Scale 50µm.

Further, we observed the transition of Brownian motion retarded LC droplets orientation from bipolar to radial configuration after addition of SDS surfactant (Fig. 4) and $_L$-DLPC phospholipids (see Fig. S7†). This matches with the results described in Fig. 1 and 3. Results mentioned above clearly demonstrates the applicability of the proposed approach for anchoring the LC droplets encapsulated polymer capsule on solid substrates via click chemistry for biosensing application.

Furthermore, to show the applicability of the proposed approach in fabrication of microfluidics sensing devices, we have anchored the LC droplets encapsulated polymer capsule on a patterned glass substrate via click chemistry. In a manner similar to scheme 1 and scheme S1, Fig. 5a shows the schematic representation for anchoring on to a patterned glass substrate using combination of micro-contact printing technique and click chemistry. Profilometer image (see Fig. S8†) clearly suggests that PDMS stamp having width of ~30 µm and height of ~1µm have been fabricated which is further used to create the patterns on glass substrate as reported in earlier reports.[29] Scanning electron microscope (SEM) image shown in Fig. 5b clearly delineates the patterns (width ~30 µm) of LC droplets encapsulated polymer capsule over a large area of substrate. We note that the formation of patterned LC droplets could not be captured by the polarized light microscopy because of the limitation of optical microscope capabilities (see Fig. S9†), SEM images were taken after drying the substrate which results in the shrinkage of anchored polymer capsules and subsequent leakages of LCs from polymer capsules (inset of Fig. 5b).

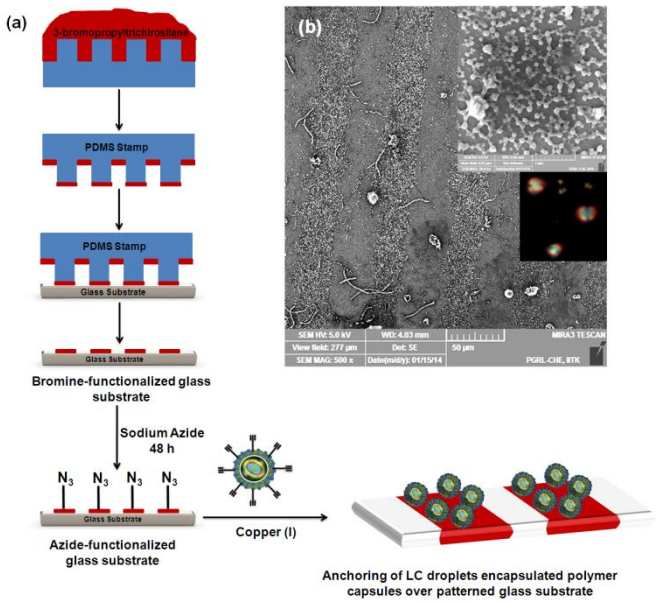



**Fig. 5** (a) Schematic representation and (b) scanning electron microscopy image of pattern anchored alkyne-functionalized LC droplets encapsulated polymer capsule on azide-functionalized glass substrate via click chemistry. PLM inset image scale 10µm.

## Conclusions

In conclusion, we have reported an approach for anchoring highly stable Brownian motion retarded LC droplets encapsulated within polymer capsules over planar substrate via click chemistry. The method is also implemented for anchoring over patterned substrates to facilitate spatial resolution and temporal detection of analyte for extended period of time. Confocal and polarized microscopy images confirm the anchoring and the sensing response upon the addition of SDS surfactant/$_L$-DLPC lipid. The anchored LC droplets are stable

over six months due to the presence of highly stable triazole linkage between LC droplets encapsulated polymer capsule and glass substrates. This approach can be potentially used in the fabrication of portable microfluidic biosensors.

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## Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, characterization techniques, FTIR spectroscopy, NMR spectra, confocal, fluorescence and polarized microscopy images, Profilometer image. See DOI: 10.1039/b000000x/

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