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Oligopeptide-decorated Liquid Crystal Droplets for Detecting Proteases

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1. Materials

Glass slides were obtained from Fisher Scientific (U.S.A). \(N,N\)-Dimethyl-\(n\)-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), glutaraldehyde (50 wt % in \(H_2O\)), \(\alpha\)-chymotrypsin (from bovine pancreas, TLCK treated to inactivate residual trypsin activity, type VII, activity \(\geq40\) units/mg) and trypsin (from porcine pancreas, Type II-S, activity = 1,000-2,000 units/mg) and cyanogen bromide (CNBr) were purchased from Sigma Aldrich. Phosphate buffer saline (PBS) was purchased from UniRegion Bio-Tech, Taiwan. Sodium cyanoborohydride (NaBH\(_3\)CN) was purchased from Acros Organics. Oligopeptide (PCT and PT) were purchased from Yao-Hong Biotechnology Inc., Taiwan. Cy3 mono-reactive dye pack for tagging PCT was purchased from GE Healthcare. Liquid crystal 4-cyano-4’-pentylbiphenyl (5CB) was purchased
from TCI (Japan). Ultrapure water was obtained by using an arium® 611 UV system (Sartorius Stedim Biotech).

2. Preparation of DMOAP-coated slides

To clean the surface, glass slides were immersed in a 5% Decon-90 solution (a commercially available detergent) for 2 h, sonicated in water for 15 min, and rinsed thoroughly with water twice. After this, the slides were dried under a stream of nitrogen. The cleaned glass slides were immersed in an aqueous solution containing 0.1% (v/v) DMOAP for 10 min, and then rinsed with copious amounts of water. DMOAP-coated slides were dried under a stream of nitrogen and heated in a 100°C vacuum oven for 15 min.

3. Critical micelle concentration (CMC) of PCT

The CMC of PCT can be estimated by using pyrene as a fluorescence probe. In short, we prepared the solutions containing 0, 1, 10, 100, 1000, 4000 μg/mL PCT and 0.6 μM of pyrene and incubated at 37 °C overnight under constant shaking. After that, the emission spectra of the solutions were recorded with the excitation at 337 nm. Intensity ratios of the third emission peak located at 383 nm (I₃) to the first emission peak located at 372 nm (I₁) were plotted against the log of PCT concentrations. By fitting the plot at low and high PCT concentrations, the CMC of PCT was estimated as the intersection of the best-fit lines.

4. Preparation of oligopeptide-decorated LC droplets

Oligopeptide-decorated LC droplets were prepared by mixing 5CB (5 μL), different concentrations of oligopeptide in 1× PBS buffer (300 μL), 500 mM NaBH₃CN in DI water (300 μL) and glutaraldehyde (1 μL) with a vortex mixer at 3000 rpm for 15 min. The mixture was left
overnight to stable and settle the LC droplets. After that, excess peptide was removed from the bulk aqueous phase by replacing the supernatant with equal volume of 1×PBS buffer twice.

5. Characterization of LC droplets

To characterize LC droplets, 2 µL of LC droplets was dispensed on the DMOAP-coated slide and allowed to settle for 5 min. The LC configuration inside the droplets was determined by using a polarizing optical microscope (Leica, Germany) in transmission mode and bright field. Each image was captured by a digital camera mounted on the microscope using 5 × objective with an exposure time of 1/80 s. Fluorescence images were captured under a Cy3 filter using the same microscope with an exposure time of 3.2 s. The fluorescence intensity of the droplets was obtained from the statistical analysis of 10 droplets from each sample using ImageJ as the software. ζ-Potential measurements of PCT-decorated LC droplets were obtained using DelsaTM nano ζ-potential (Beckman Coulter Inc., USA). The diameter of the droplets was obtained from the statistical analysis of 50 droplets from each sample using imaging software from Canon (EOS utility).

6. Protease detection by LC droplets

Different concentrations of α-chymotrypsin, trypsin and CNBr solutions were prepared in 1× PBS buffer. To detect proteases, 2 µL of LC droplets solution and 2 µL of protease solution were mixed at room temperature for 1 h then the solution was dispensed on the DMOAP-coated slide and allowed to settle for 5 min. The images of the droplets were obtained as the method mentioned above.

7. Analysis of cleaved PCT by HPLC
HPLC data were obtained by analyzing the solutions with different compositions by using a high-performance liquid chromatography (HPLC) system (Jasco, Japan) equipped with a ODS-A C18 column (10 x 200 mm ID, particle size: 5 μm) and a UV detector. Analyses were performed by UV absorbance at a wavelength of 220 nm and at a flow-rate of 3 mL/min. Solvent A was water containing 0.1% trifluoroacetic acid, and solvent B was acetonitrile containing 0.1% trifluoroacetic acid. Gradient runs were performed by using 10% to 100% solvent B over 30 min.

Fig. S1. The critical micelle concentration (CMC) of PCT was estimated by using the plot of $I_3/I_1$ obtained from the emission spectrum of pyrene as a function of logarithm of PCT concentration (μg/mL).
Fig. S2. Polarized (top row) and bright field (bottom row) images of the LC droplets prepared by different concentrations of PT when they are deposited on DMOAP-coated slide.

Fig. S3. Fluorescence images of the Cy3-labeled PCT decorated LC droplets (a) before and (b) after they were immersed in the solution containing 5 μg/mL of α-chymotrypsin for 1 h.
Fig. S4. HPLC chromatograms of (a) 1000 µg/mL PCT solution, (b) 1000 µg/mL PCT solution + 5 µg/mL trypsin, and (c) LC droplets decorated by 1000 µg/mL PCT + 5 µg/mL trypsin.

Table S1. HPLC retention time for different solution compositions.\(^a\)

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\(^a\) The incubation time is 1 h.
References: