Structural coloration from total internal reflection at microscale concave surfaces and use for sensing in complex droplets

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ABSTRACT

Complex emulsion droplets consisting of hydrocarbon and fluorocarbon oils dispersed in water have been shown to exhibit iridescent structural color via interference from total internal reflection, and the color is tunable based on the size, shape, composition, and orientation of the droplets. Our study explores the structural color properties of complex emulsion droplets and their application to colorimetric chemical sensing through the use of an α- amylase responsive surfactant solution composed of γ-cyclodextrin, Triton X-100 surfactant, and Capstone FS-30 surfactant. We aim to demonstrate proof-of-concept sensitivity of biphasic oil-in-water emulsion droplets for colorimetric sensing through the correlation of reflected structural color patterns to droplet shape and size.

Keywords: emulsions, sensors, responsive materials, structural color

1. INTRODUCTION

Structural color often arises from optical interference resulting from light interacting with nanoscale periodic structures on the scale of the wavelength of visible light[1]. Structural color is commonly observed in nature, such as in opals, peacock feathers and neon tetra fish[2]. Work by Goodling et al. has also shown recently that structural coloration can be created when white light undergoes total internal reflection (TIR) at microscale concave interfaces, such as within monodisperse Janus emulsion droplets of fluorocarbon and hydrocarbon oils dispersed in water[3]. The Janus droplet morphology is generated due to the low interfacial tension between the fluorocarbon and hydrocarbon oils, and the droplet morphology can be reconfigured by addition of surfactants that tune the balance of interfacial tensions with the water.[4] Goodling et al. demonstrated that the colors result from TIR at the hydrocarbon-fluorocarbon interface (where there is a high-to-low refractive index contrast). As a result of this mechanism, the coloration in these emulsion droplets is affected by the refractive index contrast between the liquids, droplet size, shape of the interface between the oils, and the orientation of the droplet (**Figure 1**)[3].

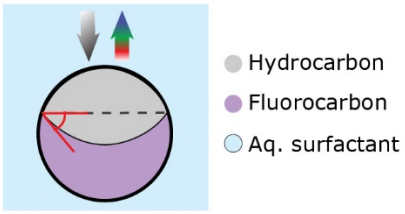

Figure 1. An illustration of the side view of a biphasic oil-in-water emulsion droplet. Hydrocarbon oil (e.g. heptane) has a higher refractive index (n=1.38) than fluorocarbon (e.g. perfluorooctane, n=1.28) enabling TIR at the hydrocarbon-fluorocarbon interface that generates reflected structural color. The angle outlined in red, along with the droplet radius of curvature, determine the geometry of the oil-oil interface, which in turn influences the possible paths of TIR. The dotted line is the three-phase contact line.

Given that these biphasic oil droplets can undergo morphological reconfiguration in response to various stimuli, such as a pH[4], light[4,5], or enzymes[6], and changes in reflected color can be associated with the droplet morphological changes[3], we aimed to explore if these emulsions could be used for colorimetric chemical sensing. A previous study by Zarzar et al. utilized interactions between the enzyme α-amylase, γ-cyclodextrin (γ-CD), and the hydrocarbon surfactant Triton X-100, among others, to develop a system that quantifies the amylase enzyme activity as a function of droplet

shape[6]. Observation of α-amylase activity is useful for the detection of physical and psychological stress in humans and animals and can signal disease states such as pancreatitis[7,8]. Broadly, this system can serve as a proof-of-concept for colorimetric chemical sensing with droplets, as in principle the droplets can be sensitized to any stimuli that would trigger a change in the balance of interfacial tensions at the droplet surface. The α-amylase-responsive surfactant is formed by mixing the Triton X with γ-CD, which form a 1:1 inclusion complex in solution[9]. This complexation renders the Triton X relatively ineffective as a surfactant. The addition of α-amylase causes the degradation of γ-CD via hydrolysis, thus freeing the Triton X (**Figure 2**) and allowing the surfactant to affect the droplet interfacial tensions and gradually change the droplet shape[6]. Presence of a fluorosurfacant, such as Capstone FS-30, is also a critical component to enabling the sensing mechanism[5]. Progress on this research is still underway, and here we report our preliminary examinations of how the reflected color of biphasic emulsion droplets can be correlated to varying concentrations of amylase, Triton X, Capstone, and γ-CD to form the basis of a droplet-based colorimetric sensing platform.

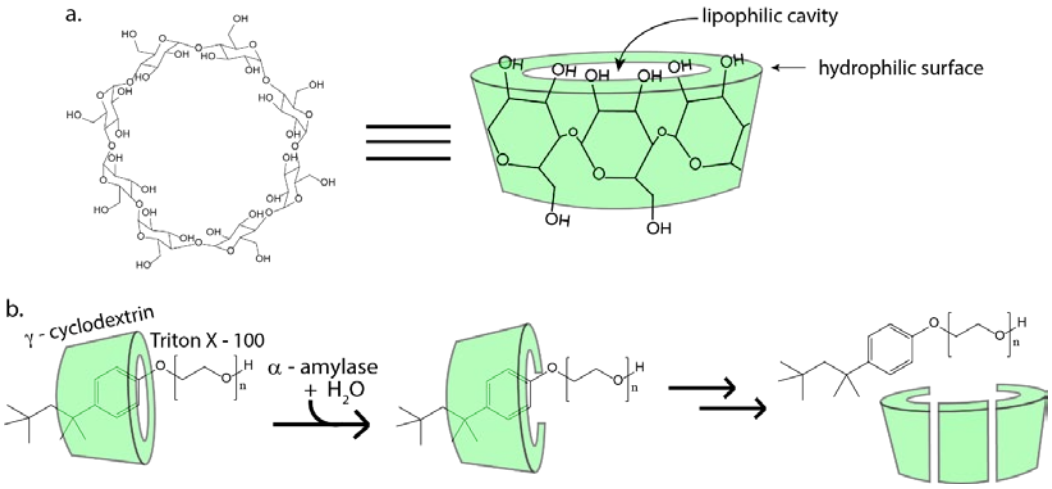

Figure 2. A summary of how the Triton X and γ-CD respond to α-amylase. **a**. γ-CD in both its skeletal and toroidal representations. The toroidal structure of γ-CD, represented in green, forms a lipophilic core and hydrophilic surface when dissolved in water.[6] **b**. A simplified scheme of γ-CD degradation in relation to its regulation of Triton X surface activity via complexation. In aqueous solution, the nonpolar region of Triton X inhabits the lipophilic cavity of γ-CD to form a 1:1 inclusion complex rendering Triton X relatively inactive as a surfactant. Ester bonds between glucose subunits of γ-CD are hydrolyzed by the enzyme α-amylase thus freeing Triton X to act in solution as a surfactant.

2. Methods

Chemicals. All chemicals were used as received. Capstone FS-30 and perfluorohexane(s) (SynQuest Laboratories); Triton X-100 and α-amylase from *Aspergillus oryzae* (Product A8220, Batch SLBV4784) (Sigma Aldrich); sodium acetate (99%) and hexane(s) (Fisher Scientific); hydrochloric acid, 1N (Ricca Chemical); γ-cyclodextrin (γ-CD) (Tokyo Chemical Industry); heptane (VWR International); perfluorooctane (TMC).

Sodium acetate buffer. The sodium acetate buffer solution was formed by adding hydrochloric acid (1N) to an aqueous sodium acetate solution (0.2 M) until the pH of the resultant solution was near a pH of 5.2. The pH was monitored using the Mettler Toledo Seven Compact s220 pH/ion meter. The sodium acetate buffer solution was then used as the standard solution for all subsequent stock solutions of Triton X-100, Capstone FS-30, γ-cyclodextrin, and α-amylase.

Droplet fabrication. Biphasic emulsion droplets were fabricated via microfluidics using a 4-inlet 100 micron channel depth hydrophilic glass microfluidics chip (Dolomite). The two inner channels were used for hydrocarbon and fluorocarbon oil flows, while the outermost channels were used for aqueous surfactant flow. Flow channels were connected to reservoirs of desired liquids via 30 inch poly ether ether ketone (PEEK) tubing of inner diameter 0.0025 inches and outer diameter 1/16 inches. Liquid flow within the microfluidics chip was controlled by the Fluigent MFCS-EZ pressure controller. Pressures ranges for the inner and outer channels ranged from 250 – 350 and 2000 – 3000 mbar respectively. Typical inner phases included combinations of heptane, hexane, perfluorooctane, and perfluorohexane, while combinations of Capstone FS-30, Triton X-100, and γ-cyclodextrin served as common outer phases.

Microscopic Imaging. All transmission photomicrographs were taken with the Nikon Eclipse Ti-U inverted microscope. Droplets orient with the denser fluorocarbon phase downward. To image the side-view of the droplets the containment dish was shaken to induce the droplets to roll onto their side. Then an image was captured with an Image Source DFK 23UX249 color camera. Photomicrographs of droplets in reflection were taken with the Zeiss Axioscope upright reflection microscope on the Axiocam 305 color camera at exposures between 5 – 30 ms.

Macroscopic Imaging. Samples for macroscopic images or videos were first prepared by filling a 35-mm petri dish lid or 24-well plate with a monolayer of emulsion droplets. All petri dishes and well plates were painted with black acrylic paint to limit reflection from the bottom layer of plastic. For small area illumination, a specific area of the droplets was exposed to a Thorlabs LED light (MWWHF2, 4,000 K, 16.3 mW) fit with a 200 µm-diameter fiber optic cable and collimating lens (CFC-2X-A). For large area illumination, an Amscope LED 50 W light with a collimating lens was used to illuminate the full sample. Images of color projections were collected by placing a hemisphere of a ping-pong ball with a three-millimeter hole in it on top of the sample and illuminating a small area of the sample. Images and videos were collected using a Canon EOS Rebel T6 DSLR camera mounted on an optical table with the light source. Phone images and videos were collected with the Galaxy Note 8 phone camera, and the phone flash was used as the only source of illumination. Angle measurements seen in text were measured using the optical table or with the Android application Bubble Level (v 3.23) of ±0.1^{o} accuracy. A diagram of color map imaging used in this experiment is shown in **Figure 3**.

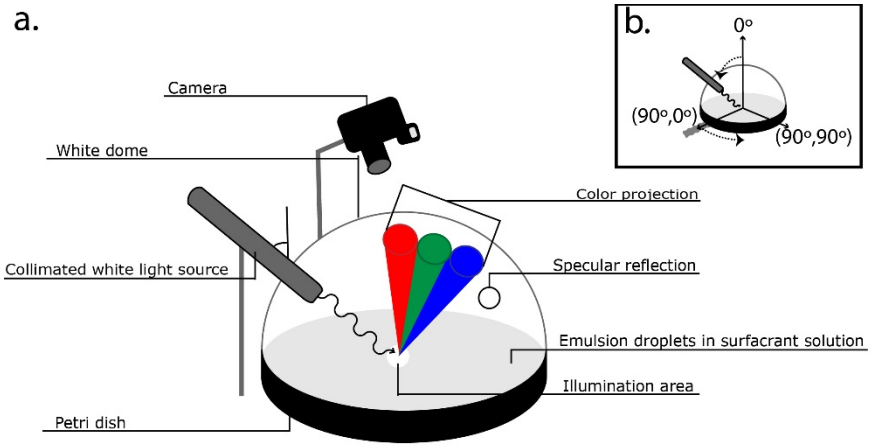

Figure 3. **a,** A schematic diagram of the color distribution mapping method, adapted from Goodling et. al.[3] A ping-pong ball hemisphere is placed on top of a petri dish containing emulsion droplets in aqueous surfactant solution. Droplets are illuminated with a white light source and colors reflect onto the dome. **b,** Diagram of spherical coordinate system used to define illumination and camera angles. Not drawn to scale.

α-Amylase Detection. Room temperature amylase transitions were prepared by placing perfluorohexane (PFH) and 1:1 heptane/hexane by volume emulsion droplets in 500 microliters of enzyme responsive surfactant solution containing Capstone FS-30, Triton X-100, and γ-cyclodextrin (γ-CD).n. Specific concentrations for each sample are given in the Figures. Samples were then exposed to concentrated 10 wt% α-amylase solution in varying volume to tune amylase activity. For α-amylase detection in a 30 ºC oven, wells of a 24-well plate were filled with 200 microliters of 10:1:10 ratio by weight of Capstone FS-30: Triton X-100: γ-CD (Capstone:TritonX:γ-CD) with Capstone being 0.2 wt% in concentration. A monolayer of perfluorooctane (PFO) and heptane emulsion droplets formed via microfluidics was then added to each well followed by 300 microliters of sodium acetate buffer and then 100 microliters of αmylase stock solutions ranging from 8.83 - 177 FAU/L. Experiments were conducted at 30 ^{o}C using an oven to regulate the temperature. Experimental setup is shown in **Figure 4.**

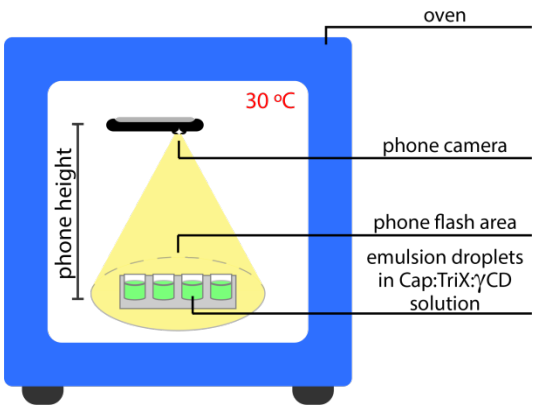

Figure 4. A simplified diagram of the method used to record video of the amylase transition times under heated conditions. A cell phone was suspended 24 centimeters above a 24-well plate of perfluorooctane/heptane emulsion droplets in Capstone, Triton X, γ-CD solution. After adding α-amylase, video of the amylase transition from a 0° illumination and camera angle was recorded using the phone camera and flash while inside an oven with ambient temperature of 30 °C.

3. RESULTS AND DISCUSSION

Color distribution and color position as a function of droplet shape. Droplets of varying shape were created by adding increasing amounts of Triton X-100 to droplets stabilized with Capstone and imaged with the method shown in **Figure. 3.** Side view images of droplets and the color distributions are shown in **Figure 5**. Changes in the position of the colors are associated with the change in the shape of the emulsion droplet, largely as a function of the geometry of the interface between the two oils as expected given the findings in Goodling et al.[3] When emulsion droplets were placed in the enzyme responsive surfactant solution (containing Triton X, γ-CD and Capstone) and exposed to α-amylase, the full color band sweeping motion is visible as the droplet shape changed continuously (**Movie S1**).

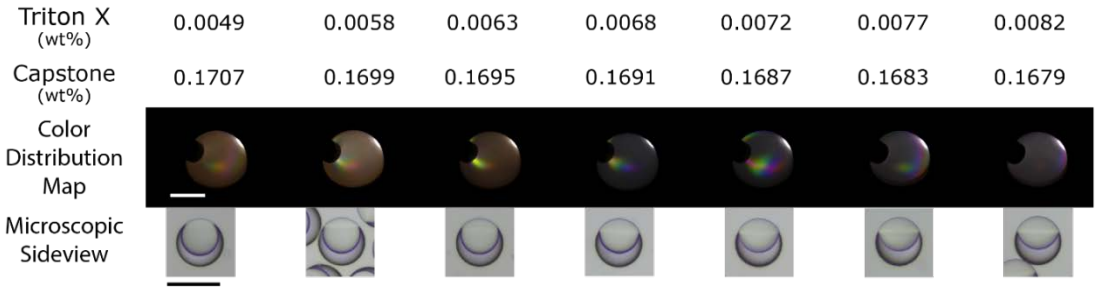

Figure 5. Images of color distributions (scale bar: 2 cm) accompanied by corresponding microscopic sideview images (scale bar: 50 micron) of droplets containing perfluorohexane and a 1:1 mixture of hexane and heptane by volume beginning in 0.175wt% Capstone and continuing with additions of Triton X. From left to right, the fraction of Triton X increases causing emulsion droplets change morphology so as to create more hydrocarbon-water interfacial area. This change in droplet shape resulted in changes in the colors projected onto a ping-pong ball hemisphere. Illumination angle = 40°.

Color variation as a function of droplet size and illumination angle. In accordance with the results seen in Goodling et al., coloration varied by droplet size and shape. Upon addition of amylase to a droplet solution containing Capstone, Triton X, and γ-CD, the droplet shape continuously changed over time as more Triton X was released; as the droplet shape changed, so did the reflected color distribution, as seen in **Movie S2**, which shows side-by-side the color transitions occurring at a 40° illumination angle for two different sizes of droplets (45 micron and 57 micron diameter). **Figure 6** shows bright field transmission images of the droplets as well as frames from **Movie S2** showing samples of the most colorful state. Dependence of color positions on illumination angle is also expected and was observed to be the case in **Movie S3**, where upon changing the illumination angle from 40° (seen in **Movie S2**) to 0° illumination (**Movie S3**) shows a different color pattern and a difference in the origins of the sweeping motion of the colors.

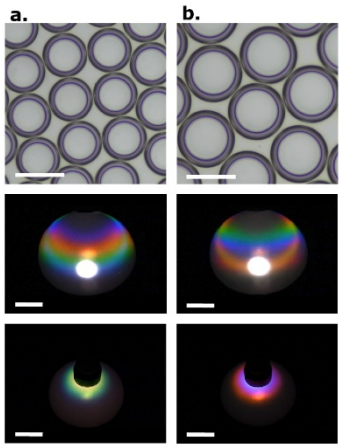

Figure 6. A comparison between droplets of two sizes and their influence on the color distributions. Images in (**a**) correspond to emulsion droplets that are 45 microns in diameter, while images in (**b**) correspond to droplets of a 57 micron diameter. Top row images are microscopic bright field transmission images of PFH and 1:1 hexane/heptane by volume emulsion droplets submerged in 0.2 wt% Capstone in the two sizes specified (scale bars: 50 micron). Middle row images are snapshots (from **Movie 2**) of color distribution maps taken while the emulsion droplets are undergoing morphological changes triggered by amylase activity. Illumination angle = 40^{o} (scale bars: 1 cm). Bottom row images are also snapshots (from **Movie 3**) of color distribution maps of emulsion droplets taken while the droplets are undergoing an amylase-induced morphological transition (scale bars: 1 cm). Illumination angle = 0^{o}.

The comparatively "simpler" pattern of the colors for 0^{o} illumination angle (e.g. a circular ring of color around the light source as seen in **Figure 6**) may make it a preferable illumination angle for colorimetric sensing. At 0^{o} illumination angle, color changes are qualitatively less variable, shorter in duration, and remain vibrant throughout most of the transition. There are benefits to illuminating and viewing the sample both at approximately 0^{o} (e.g. using the retroreflection) as this is a typical imaging configuration, such as in microscopes or when using cameras with a flash, as we have begun to explore. These aspects make tracking colorimetric changes in one area more straightforward, but it may come at the price of lower sensitivity. We did not perform a quantitative analysis to determine if the limit of detection or sensitivity is superior with a 0^{o} illumination as compared to other angles.

Phone imaging as a means of detection. We aimed to determine if a simple cell phone camera with a flash could be used to colorimetrically detect droplet changes in response to amylase activity. We conducted all phone imaging with the phone held at a 0^{o} angle and the phone LED served as the illumination source. Static phone images were taken with the phone remaining parallel to the sample and color was compared to microscopic reflection images of the emulsion droplets, as seen in **Figure 7**. Coloration found in phone images closely resembled the color reflected from near the three-phase contact line of emulsion droplets as seen in a reflection microscope. A cell phone video of the droplet color change upon addition of amylase is shown in **Movie S4**. This method may allow for amylase detection by recording the amount of time it takes for emulsion droplets to change colors (e.g. between images marked with an asterisk in **Figure 7**), or from colored to colorless. Given that the specific colors reflected vary based on droplet size and volume ratio of oil in each droplet[3], if specific color changes are desired for sensing then each droplet morphology would need to be calibrated. It is possible that using a change from colored to colorless (or vice versa) would yield more consistency between samples.

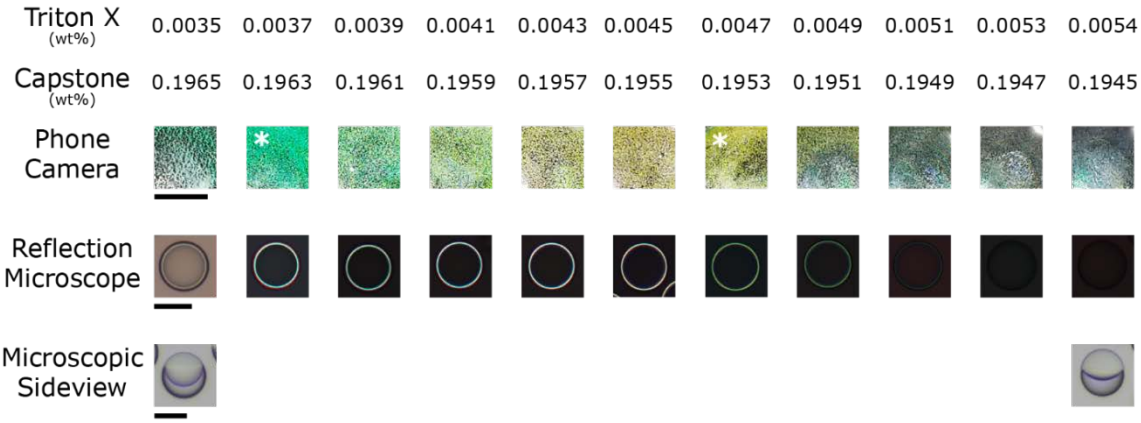

Figure 7. Correlation of droplet shape of PFH and 1:1 volume ratio heptane: hexane drops to reflected colors imaged by phone and a 10X magnification reflection microscope. Droplets began in 0.2 wt % Capstone and Triton X was added to yield the concentrations stated to change droplet shape. Top row images were captured by phone camera 18 inches above and held parallel to the sample (light from phone LED flash, illumination angle 0º). Scale bar: 2 cm. Asterisk markers were placed on images with most saturated coloration and serve to mark possible stages for the beginning and end points of amylase transition tracking. The middle row of microscopic images were collected via reflection microscope where coloration may be seen to radiate from near the three-phase contact line of emulsion droplets Scale bar: 50 micron. Coloration in this ring resembles coloration exhibited macroscopically in phone images in the top row. Bottom row images exhibit a microscopic sideview of emulsion droplets at the low and high concentration of the Triton X concentration range tested. Scale bar: 50 micron

Detection of amylase activity. α-Amylase-induced color change (defined from no color to first instance saturated coloration) at 30 ^{o}C yielded an inverse relationship between enzyme activity and wait time between color states. Higher enzyme activities correlated to the shorter times. Use of the perfluorooctane and heptane drops in 10:1:10 Capstone:Triton X:γ-CD by weight starting aqueous surfactant (0.08 wt% concentration of Capstone) system at 30 ^{o}C for a range of amylase activity over time is displayed below in **Figure 8**. In order to further calibrate the accuracy and the limit of detection of the emulsion droplets as well as decrease color change times, trials of the amylase enzyme sensing should be conducted at 37 ^{o}C between activities from about 10 to 200 FAU/L in order to derive a continuous function for the relationship between enzyme activity and droplet transition time. This higher temperature should significantly speed the enzyme kinetics while decreasing the wait time for α-amylase detection.

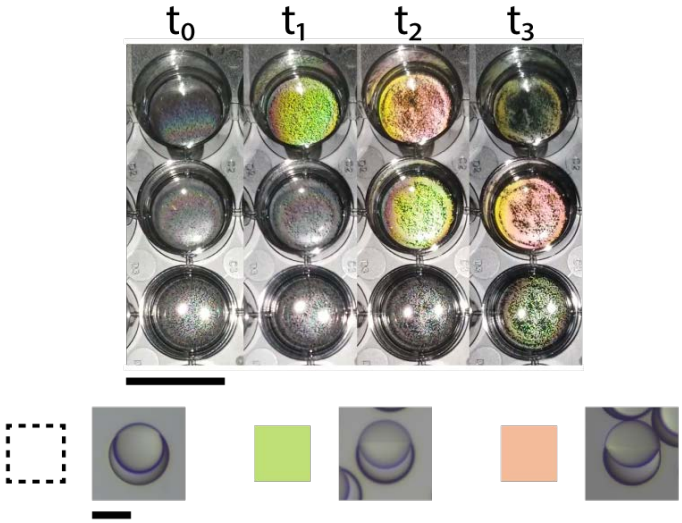

Figure 8. Top row images each display the same three wells in a single column of a well plate used for α-amylase time trials at 0^{o} illumination and 0^{o} camera angle (scale bar: 2 cm). For the duration of the time trials, wells were maintained in an oven at an ambient temperature of 30 ºC. Each well contained PFO and heptane emulsion droplets in 0.08 wt% Capstone: 0.008 wt% TritonX: 0.08 wt% γ-CD starting surfactant solution prior to the addition of amylase. The leftmost image at t_0 indicates

the starting state of the emulsion droplets just as amylase was added to the sample (t_0 = 0 minutes). The top well was introduced to amylase of 176.6 FAU/L, the middle well was introduced to 100 FAU/L, and the bottom well was introduced to 66.2 FAU/L. From left to right, images display the change in coloration of emulsion droplets in all three wells with increasing time (t_1 = 49 mins, t_2 = 64 mins, and t_3 = 92 mins). Bottom row images compare observed coloration and respective approximate microscopic side view images of emulsion droplets at that coloration state (scale bar: 50 micron).

4. CONCLUSION

A comprehensive range of coloration in fluorocarbon-hydrocarbon emulsion droplets was explored via enzyme-induced changes in interfacial tension and droplet shape. Future studies will aim to correlate droplet shape with color band position by performing more detailed static image analysis at smaller concentration increments of Triton X-100 and Capstone FS-30 ratios. We demonstrated proof-of-concept that the droplet-based colorimetric detection can serve as a simple platform using a portable mobile phone for imaging with illumination from the camera flash. Future studies will examine a broader range of α-amylase activities and correlation of the color change rates with enzyme activity, which would enable estimation of the limits of detection of the emulsion droplet system as well as sensitivity and accuracy.

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5. SUPPLEMENTARY MOVIE DESCRIPTIONS

Movie S1. Color distribution projection of perfluorohexane and 1:1 volume heptane/hexane emulsion droplets after α-amylase was added to the drops in 5:1:15 Capstone:TritonX:γ-CD (0.2 wt% Capstone) aqueous solution. Light incidence: 40^o. Video playback at 25x speed.

Movie S2. Side by side view of color distribution projections of perfluorohexane and 1:1 volume heptane/hexane emulsion droplets after α -amylase was added to the drops in 5:1:4 Capstone:TritonX:γ-CD (0.2 wt% Capstone) aqueous solution. Left: Droplets in sample are 45 μm in diameter Right: Droplets in sample are 57 μm in diameter. Light incidence: 45^o. Video playback at 25x speed

Movie S3. Side by side view of color distribution projections of perfluorohexane and 1:1 volume heptane/hexane emulsion droplets after α -amylase was added to the drops in 5:1:4 Capstone:TritonX:γ-CD (0.2 wt% Capstone) aqueous solution Left: Droplets in sample are 45 μm in size Right: Droplets in sample are 57 μm in size. Light incidence: 0^o. Video playback at 25x speed.

Movie S4. Phone video of perfluorohexane and 1:1 volume heptane/hexane emulsion droplets after α-amylase was added to the drops in 5:1:4 Capstone:TritonX:γ-CD (0.2 wt% Capstone) aqueous solution. Phone angle: 0^o. Phone height: 10 inches above sample. Video playback at 30x speed.

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