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Supplementary Information

Colorimetric response in polydiacetylene at the single domain resolution by hyperspectral microscopy

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Materials and methods

Polydiacetylene preparation

10,12-Tricosadiynoic acid (TRCDA, CAS No: 66990-30-5, Sigma-Aldrich, Inc., USA) was dissolved in chloroform (Guaranteed Reagent, CAS No: 66990-30-5, FUJIFILM Wako Pure Chemical Corporation, Japan) at the concentration of 25 mg/ml and was used within 4 months. The TRCDA solution was drop-casted on a glass coverslip that is pretreated with an oxygen plasma cleaner (Plasma Cleaner PE25, Plasma Etch, Inc., USA). After chloroform was evaporated completely, the TRCDA was polymerized in a UV box (UVP Crosslinker CL-3000, Analytik Jena US, USA) at the UV dose of 0.25 J/cm^2.

Polydimethylsiloxane(PDMS) preparation

Silicone Elastomer Base (The Dow Chemical Company, USA) and Silicone Elastomer Curing Agent (The Dow Chemical Company, USA) were mixed at 10:1 and were poured into plastic plates (ND90-15, AS ONE Corporation, Japan) with a thickness of around 1 mm. These plates were placed in an oven (ONW-600SB, AS ONE Corporation, Japan) at 40 °C for 24 hours to solidify. Once cured, they were cut into small squares with a dimension of 20 × 20 mm^2 and a hole with the diameter of 4 mm was created in the middle of these PDMS membranes.

Heat activation of PDA

The ring-shaped PDMS described above was placed on the PDA-assembled glass coverslips. This PDMS is to enable heating the PDA sample on the optical microscope stage without moving, whereas the imaging was performed at the center through the hole. After imaging the PDA in the blue form both in the crystal structure and amorphous structures, a tip-shaped hot iron (Sure-ishizaki, Japan) was positioned on the PDMS at 240 °C for 1 min. This procedure turned the blue PDA into red.

pH activation of PDA

In the pH-induced experiment, 80 μL of 1M NaOH (FUJIFILM Wako Pure Chemical Corporation, Japan) was drop on the PDA sample made by drop-casting and incubating for 30 minutes.

Hyperspectral microscope

The hyperspectral images were taken by a hyperspectral microscope (CytoViva, Inc., USA), where an upright optical microscope (Olympus Corporation, Japan) is coupled with a ImSpector V10E (Specim, Spectral Imaging Ltd., Finland), with a 60x objective lens with NA 0.7 (Olympus Corporation, Japan). 696 scan lines were applied for acquiring images with exposure time of 0.25 s for each line, the illumination intensity was 58.3%. Imaging with the hyperspectral microscope produces a dataset called “datacube”, where the image is constructed by 696 × 696 pixels in *x* and *y*, and the third axis represents the wavelength of the transmitted light, which has the spectral resolution of 1.3 nm (466 bands between 400 and 1000 nm). When we compare the blue and red PDA signals, TurboReg plugin in Image J was used to superpose their positions as heating procedure sometimes shifted the sample position slightly. ENVI v4.8 (L3Harris Geospatial Solutions, Inc., USA) with a CytoViva plugin was used to analyze and present the hyperspectral data.

Spectral angle mapper classification in ENVI v4.8 software

Here, we describe the detail of the data analysis using ENVI v4.8 software. The spectral angle mapper (SAM) was employed to categorize the distribution of the PDA spectra. In brief, SAM stores the spectrum information from each pixel in a form of a multidimensional vector, which consists of the intensity values at N wavelength ($I_{\lambda 1}, I_{\lambda 2}, \ldots$). When two vectors (spectra) have completely different angles in the N dimensional space, these spectra will be categorized as "different", whereas when the angle difference is smaller than the designated threshold (tolerance) they will be considered into the same category even if their lengths are different. In other words, any spectra, where their intensities are the multiplication of a certain spectrum, will be categorized the same. This allows the effect of the sample thickness to be excluded from the spectral map. The exact image analysis procedure is as follows. First, the control datacube (the transmission datacube from a glass coverslip without PDA) was subtracted from the sample datacube (PDA on glass) to eliminate the signals that come from anything in the optical pass besides PDA such as glass coverslips and optical lenses. This converts the transmittance into absorption. The SAM was performed on the absorption datacubes with the threshold value at 0.1 radian to set the tolerance of the mapping. As an output, the SAM creates a color map, showing the spectral distribution, where each spectrum is color-coded.

Data processing in MATLAB

The obtained color map was first converted into RGB values. Next, these RGB values were converted into a grayscale. This enabled the transformation of the "color-code" into the "grayscale number-code". Blue-to-red transition was tracked using this grayscale number-code by pixel-by-pixel. Finally, the probability of a certain transition (e.g. blue grayscale number xx → red grayscale number xx) was expressed as percentage and they were plotted as a transition map in Figure 2 and 3.

Supporting figures

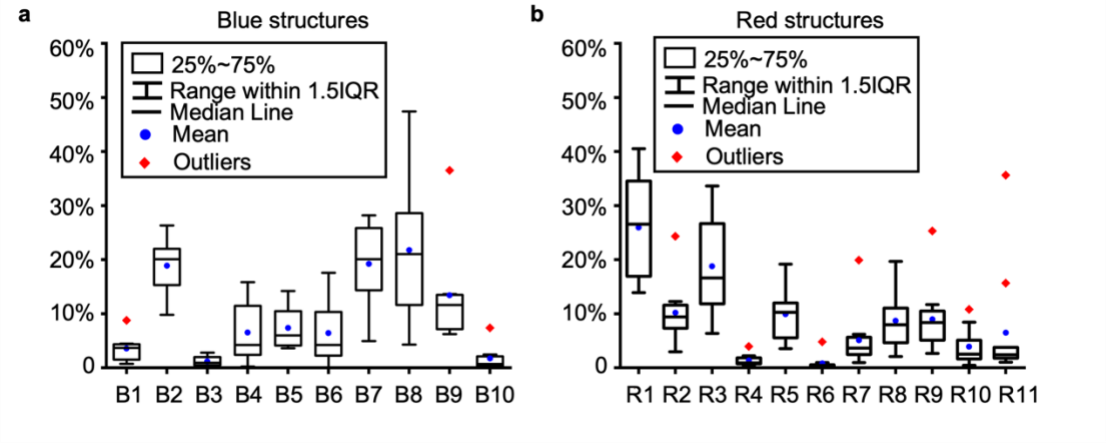

Figure S1.The structure distribution of blue and red PDA.

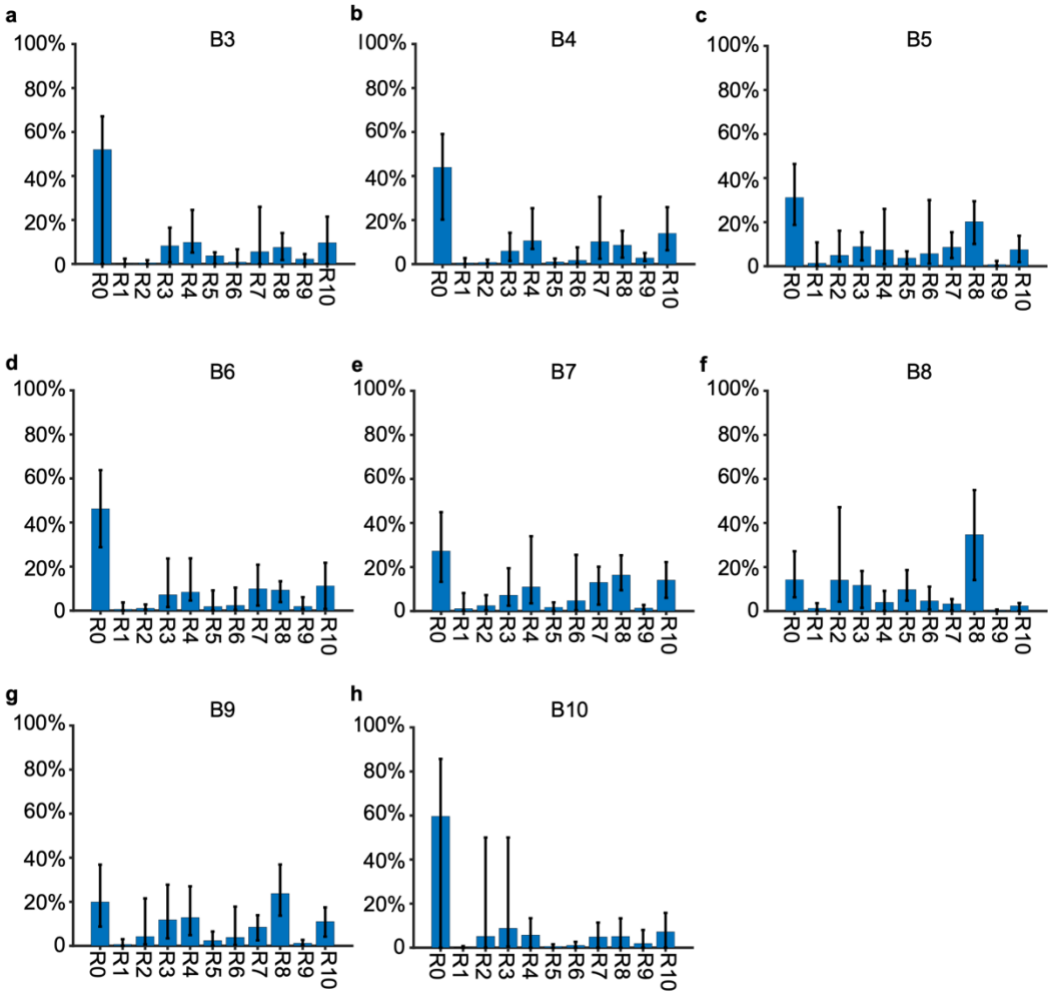

Figure S2. The pixel fraction of B4-B10 that transferred into different red structures (R0-R10).

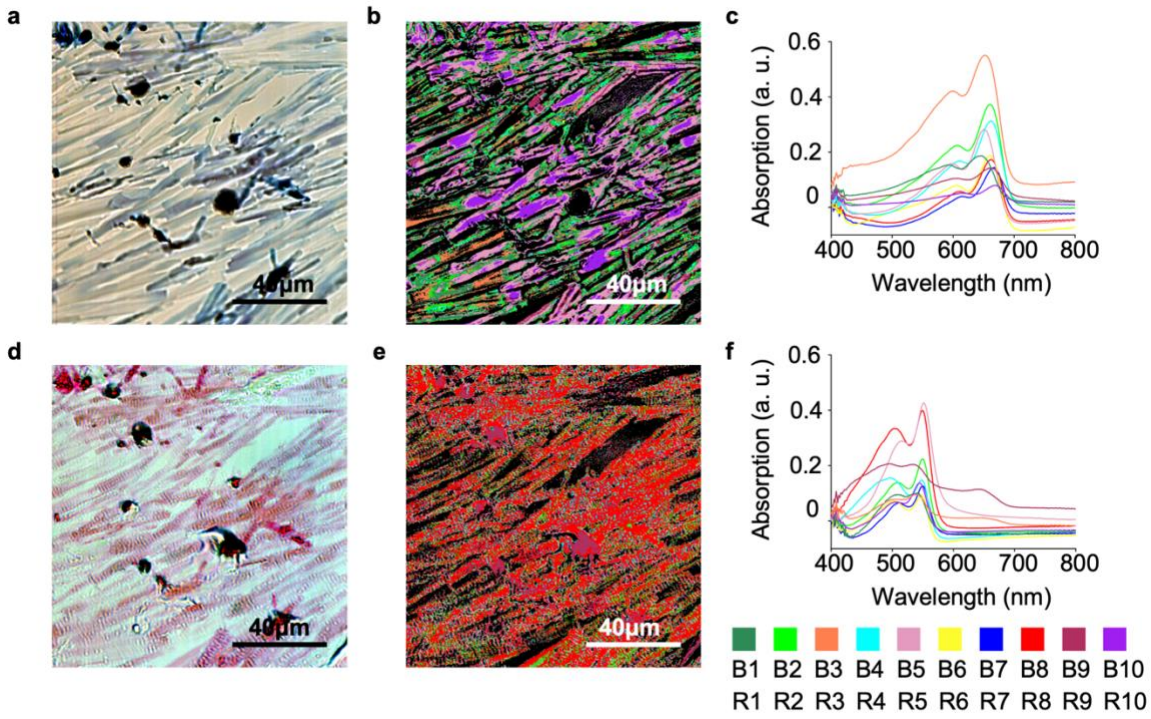

Figure S3. Thin PDA samples stimulated by heat.

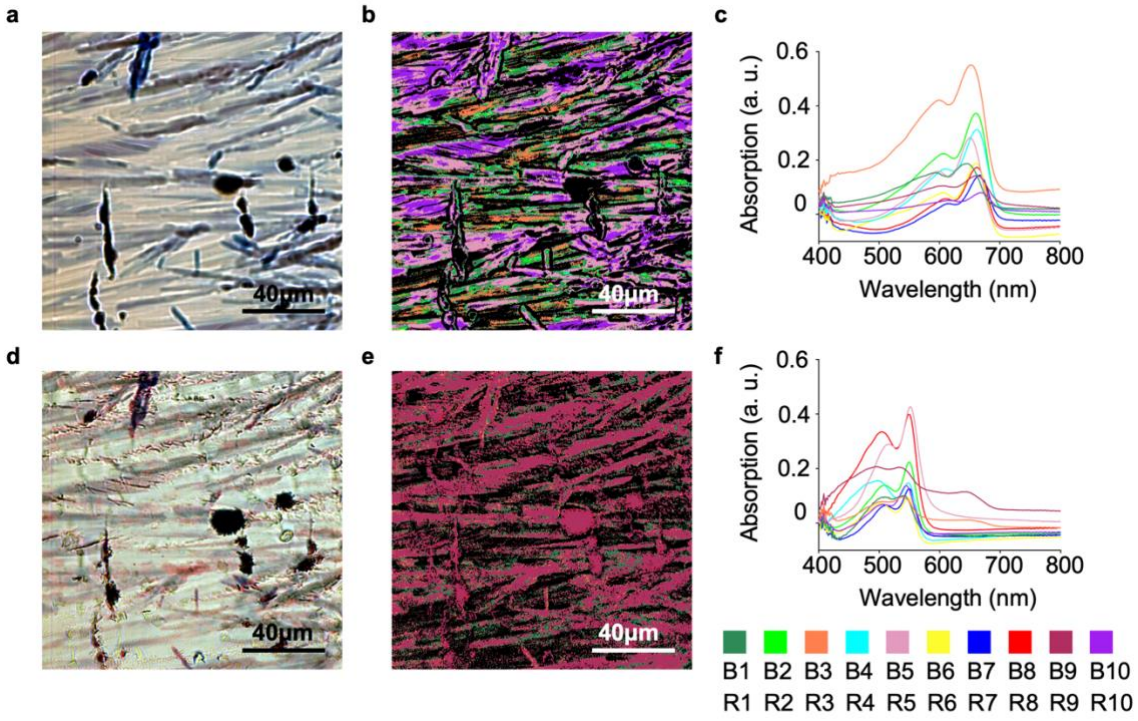

Figure S4. Thin PDA samples stimulated by pH.

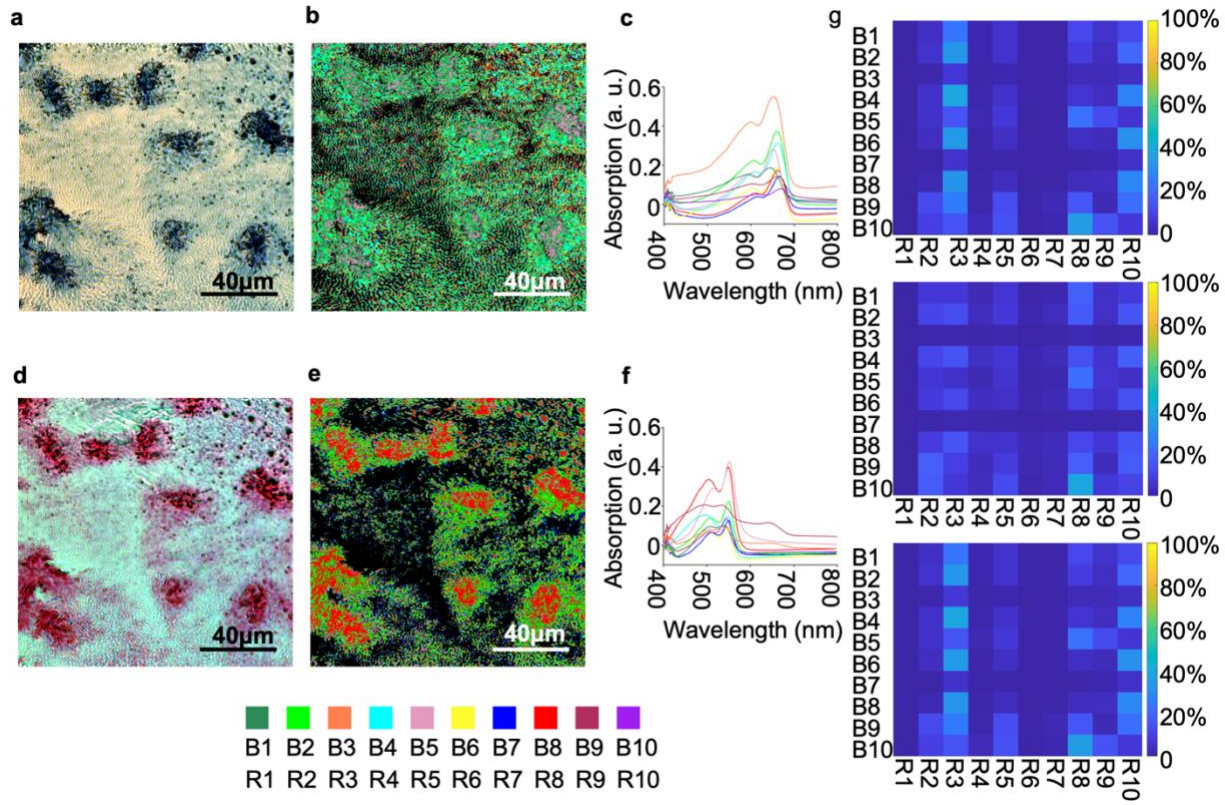

Figure S5. Amorphous PDA stimulated by heat.

Table S1. Average domain sizes

	1	2	3	4	5	6	7	8	9	10	Average
Blue domain (μm²)	0.27	0.93	0.13	0.27	0.51	0.39	0.88	2.11	0.55	0.20	0.62
Red domain (μm²)	0.13	0.27	0.28	0.22	0.29	0.21	0.26	0.61	0.08	0.23	0.26