Supplementary Information

# Quantitative and anisotropic mechanochromism of polydiacetylene at nanoscale

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# Materials and methods

## Storage of diacetylene monomers

10,12-Tricosadiynoic acid (TRCDA, CAS Nr.: 66990-30-5, abcr GmbH, Germany), 10,12-pentacosadiynoic acid (PCDA, CAS Nr.: 66990-32-7, Sigma-Aldrich, Germany) and 5,7-docosadiynoic acid (DCDA, CAS Nr.: 178560-65-1, Toronto Research Chemicals, Canada) were purchased as a powder, aliquoted and stored as following. They were dissolved in high-purity chloroform (CAS Nr.: 67-66-3, Fisher Scientific UK Limited, United Kingdom), aliquoted into glass vials and placed under vacuum for 24 h to evaporate the chloroform. Next, they were resuspended with a mixture of 96% cyclohexane (CAS Nr.: 110-82-7, Sigma-Aldrich Chemie GmbH, Germany) and 4% ethanol (CAS Nr.: 64-17-5, Fisher Scientific UK Limited, United Kingdom) solution and placed under vacuum on dry ice for 72 h in order to reduce crystal formation, thus avoiding the unwanted polymerisation during the storage. These aliquoted vials were filled with nitrogen gas (Nitrogen 4.5, PanGas, Lot: 756 311-00179757, Switzerland) and stored in a freezer ($T$ = - 26 °C) until use.

## Polydiacetylene preparation

Right before use, these aliquots of TRCDA, PCDA and DCDA were dissolved in high purity chloroform at 25 mg/ml for TRCDA and PCDA, and at 10 mg/ml for DCDA. The Langmuir-Blodgett trough (Kibron Microtrough XS, Kibron, Finland) was cleaned twice with acetone, ethanol and Milli-Q water and a test compression was made without lipids to ensure there is no contamination left at the water-air interface. The trough was kept at a constant temperature during the compression and film transfer (TRCDA: $T$ = 18 °C, PCDA: $T$ = 30 °C, DCDA: $T$ = 29 °C). These temperatures were empirically chosen to form PDA crystals with heights of around 13 nm to have comparable samples with all of the monomers. The diacetylene monomers dissolved in chloroform were spread on the air-water interface with a Hamilton glass syringe and were left for 20 min to evaporate the chloroform. The films were compressed at a rate of 7 mm/min for PCDA and DCDA and 20 mm/min for TRCDA to a surface pressure of 20 mN/m (TRCDA), 15 mN/m (PCDA) and 10 mN/m (DCDA), then transferred

vertically to a glass slide treated with an oxygen plasma cleaner (100 Plasma System, PVA TePla, Germany) for 30 min at a transfer rate of 1 mm/min. The transferred films were placed under vacuum overnight. They were polymerised under nitrogen in a UV-box at doses of 25 mJ/cm$^2$ for TRCDA and PCDA, and 3375 mJ/cm$^2$ for DCDA.

## Atomic force microscopy

Atomic force microscopy (AFM) measurements were carried out with a JPK NanoWizard 3 instrument (JPK Instruments, Germany) coupled with an inverted fluorescence microscope (see the section of the brightfield and fluorescence microscopy for the details), placed on an anti-vibration isolation platform (halcyonics_i4, Accurion, Germany) in an acoustic enclosure (JPK Instruments, Germany). The air humidity within the enclosure was kept at 5%. Fresh gold-coated n-type silicon cantilevers were used (HQ:CSC37-Cr/Au, "B" cantilever, tip radius: 35 nm, $k$ = 0.3 N/m, Mikromasch, Bulgaria) for each measurement. The measurements were carried out in contact hover mode (i.e. the tip is in contact only in the trace direction and hovers on the way back for the retrace) with 300 nm hover offset, at 0° scan angle and 0 µm offset. Areas of 50 µm x 100 µm were scanned at 512 x 1024 pixel resolution, 4 µm/s scan speed and 10% overscan, 50 Hz Igain and 0.001 Pgain. The setpoints were increased during the scan after each 100 lines (e.g. 20, 40, 60, 80 and 100 nN).

## Friction force microscopy based on wedge calibration method

Friction force microscopy, or lateral force microscopy is a mode of scanning probe microscopy, invented in 1987[1]. AFM registers the laser deflections reflected at the cantilever in two main directions, vertically (i.e. a bend of the cantilever normal to the scanning plane) and in lateral direction (i.e. a twist of the cantilever). The direct readouts of these values detected at the position sensitive detector are in volts. In contrast to the vertical deflection, where the laser deflection can be easily converted into forces by the cantilever spring constant ($k$), the lateral deflection requires an additional calibration procedure as the accurate extraction of torsional bending constant is more cumbersome. In brief, in the wedge calibration method[2, 3] laser deflection is recorded while the tip is scanning wedges (slopes). These deflections are

correlated with the estimated lateral forces that the slope applied to the tip based on force-balancing equations for obtaining a calibration factor $\alpha$. Based on this calibration factor, one can convert lateral deflections into lateral forces. In the present work, we used this method occasionally for confirming the accuracy of the calibration, whereas a simplified method that we have established before[4], where we estimate $\alpha$ from the vertical tip bending constant $\alpha$ = 14.74 x $S_n$ x $k$ was used for daily experiments.

## Compensation of a lack of loop offset in the hover mode

Using the hover mode introduces a minor technical issue in the interpretation of the friction force microscopy. In AFM, the absolute reading of the laser deflection values is not accurate as it contains some offsets that come from e.g. laser drifts. In the standard friction force microscopy, one uses "loop offset", which is a mid value of lateral deflections between trace and retrace on glass, to correct this offset[4]. However, hover mode lacks the data from retrace, which hinders the determination of the loop offset. To compensate this, we use the lateral deflection values on glass from the trace in hover mode and adjust each scanning line in such a way that this lateral deflection on glass becomes the value estimated from the separately-measured friction coefficient between the tip and the glass substrate.

To obtain the friction coefficient between the tip and the glass, after scanning the PDA samples in hover mode, a second scan was carried out at the very same area. All the scan parameters were the same as described above except that the hover mode was turned off (i.e. the AFM registers both trace and retrace during the scan). The mid lateral deflection value between the trace and the retrace scanning lines on glass allows for the accurate determination of the loop offset ("zero lateral deflection")[4], thus the lateral deflection and the lateral force. Once the lateral force was obtained, the friction coefficient ($\mu$) between the tip and the glass substrate can be calculated with the following relation.

$$F_{//} = \mu \times F_{\perp,} \qquad \text{(eq. 1)}$$

where $F_{//}$ is lateral and $F_{\perp}$ is vertical forces relative to substrates. This friction coefficient allows us to estimate lateral forces on glass at a given vertical force for each sample, which we used to adjust each scanning line taken by the hover mode.

## Brightfield and fluorescence microscopy

Brightfield and fluorescence images were taken with a Nikon Eclipse Ti (Nikon, Japan) inverted fluorescence microscope with an oil immersion objective (Objective Plan-Apochromat 60 x / 1.40 Oil DIC, Nikon, Japan) and a monochrome digital camera (DS-Qi2, Nikon, Japan) at a resolution of 4908 x 3264 pixel. Brightfield images were taken with white light LED (CoolLED pE-100, UK) as light source, with 200 ms exposure time, 1.0 x gain and 3.0 illumination power. Fluorescence images were recorded using a solid-state white-light excitation source (Sola SE 5, lumencor, USA) coupled with a TRITC filter cube (543 / 22 nm emission, 562 nm dichroic mirror, 593 / 40 nm barrier filter, SemRock BrightLine, USA) at 500 ms exposure time, 1.0 x gain and 15% illumination power.

## Fluorescence light source calibration

To compare the fluorescence images recorded on different days, the fluorescence intensity has to be calibrated to avoid the error that comes from the light intensity fluctuation. For this purpose, a red autofluorescent plastic slide (Chroma, USA) was imaged at 5 ms exposure time, 1.0 x gain and 15% illumination power after each measurement and the intensity variation was used to correct the data if necessary.

## Thermochromic experiment and the determination of transition temperatures

In order to determine the thermochromic transition temperatures of the different PDA, Langmuir-Blodgett films of TRCDA, PCDA and DCDA were heated on a heating plate (IKA RH Digital, IKA, Germany) at 50, 55, 60, 65 and 70°C, while a video was recorded simultaneously to capture the colour transition with a mobile phone (Huawei Psmart+, 1080p, 30 fps). We performed this in-situ color monitoring because the thermochromism of DCDA is reversible

at lower temperatures[5]. Snapshots from the movie (1 minute after switching the hotplate temperature) were shown in Figure 3 after adjusting the contrast via imageJ.

## Data processing in MATLAB

The recorded AFM and optical images were processed in a home-written script in MATLAB. The main steps are described in the following:

- AFM height: Background corrections and binarization of the AFM height image in order to create a master mask used for the determination of the pixels covered with PDA.
- Lateral forces: Correction of the baseline by the use of the $\mu$ value separately calculated with non-hover mode as described previously. To convert the lateral deflection to lateral force, the lateral force calibration parameter $\alpha$ is calculated with a simplified method as the following[4]:

$$\alpha = 14.74 \times S_n \times k, \qquad \text{(eq. 2)}$$

  where $k$ is the spring constant of the cantilever and $S_n$ is the vertical sensitivity of the cantilever.
- Fluorescence images: Alignment and background removal of the fluorescence images recorded before and after the scan were performed in order to calculate the fluorescence increase in each pixel ($\Delta$ fluorescence).
- Alignment of all of the images: We aligned the $\Delta$ fluorescence, AFM height, lateral force and vertical force images to the brightfield image for the superposition, as brightfield images typically yield the highest contrast, thus the algorithm worked in the most robust manner. First, the fluorescence images were aligned to the brightfield image. Next, the brightfield image was aligned to the AFM master mask, establishing the link between the optical and AFM data.
- Determination of the inner and edge regions of the PDA film: The edge of a PDA island is defined as the 3 outermost pixels (145 nm).
- Generating plots (e.g. Figure 1h-j): $\Delta$ fluorescence, lateral force, vertical force values in each 2D map were correlated pixel by pixel and binned for the plots. Binning parameters were as follows. In Figure 1h, the maximum and minimum measured lateral forces (typically in the range of 0 – 400 nN) were divided into 1000 bins, where in each bin $\Delta$

fluorescence was averaged and the standard deviation was added as an error bar. In Figure 1 i, j, vertical force was binned based on the setpoints. For example, $\Delta$ fluorescence that corresponds to the vertical forces between 20 – 30 (nominal setpoint: 20nN) was averaged and plotted at vertical force = 20 nN with standard deviation as an error bar.

## Analysis in Figure 3

The fluorescence intensity in Figure 3 is normalized by the fluorescence from each PDA sample when they were completely activated by heat as follows. This enables the quantification of the fraction of PDA that has been activated by AFM scanning, allowing the comparison between different PDAs despite variations in average heights and polymerization degree.[6] After scanning, the PDA samples were heated at 90 °C for 5 min to induce the complete blue-to-red transition. Three fluorescence images were taken from regions with a similar morphology and thickness to the scanned area. Their average fluorescence intensity was used to normalize the $\Delta$ fluorescence in Figure 3.

# Supporting figures

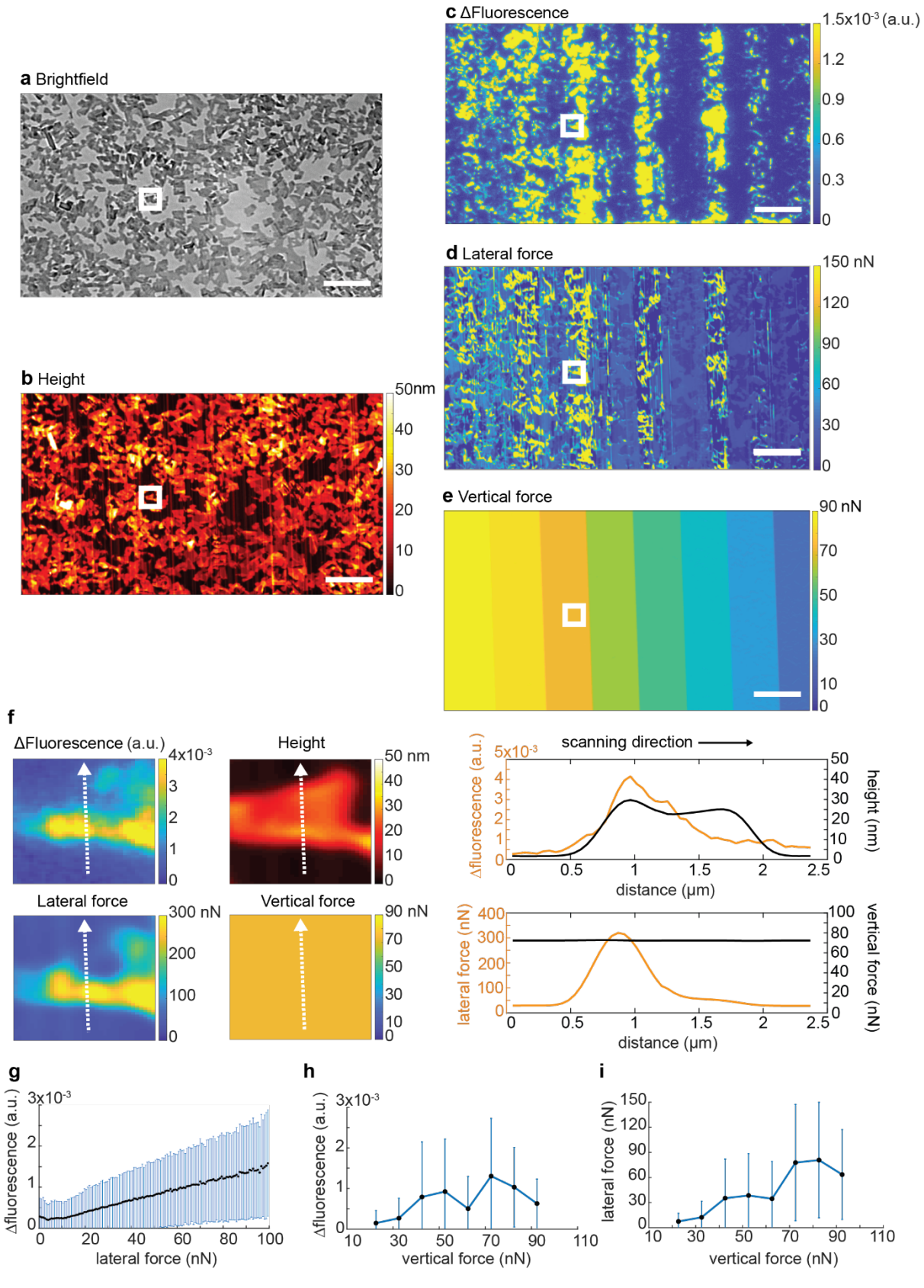

Figure S1. Characterization of 10,12-pentacosadiynoic acid (PCDA) Langmuir-Blodgett films by lateral force microscopy coupled with fluorescence microscopy. a, Brightfield microscopy image. b, AFM height. c, Fluorescence increase map. d, AFM lateral force map. e, AFM vertical force map. The scale bars show 10 µm. f, Zoom-in images of the white squares in images (b-e) and cross-sections along the white dotted arrows. Note that the white arrows in the zoom-in images showing the scanning direction are slightly tilted due to the alignment of the AFM images to optical images. These Δ fluorescence, Lateral force, Vertical force maps were correlated pixel by pixel and binned to generate plots in g-i; g, Fluorescence increase vs. lateral force, h, Fluorescence increase vs. vertical force, i, Lateral force as a function of the applied vertical force. The error bars show the standard deviation in each bin. Lateral force microscopy experiments were carried out in contact hover mode, with increasing setpoints (i.e. vertical force) after each 100 lines (20, 30, 40, 50, 60, 70, 80 and 90 nN) at 4 µm/s scanning speed. Areas of 50 x 100 µm were scanned at a resolution of 512 x 1024 pixel.

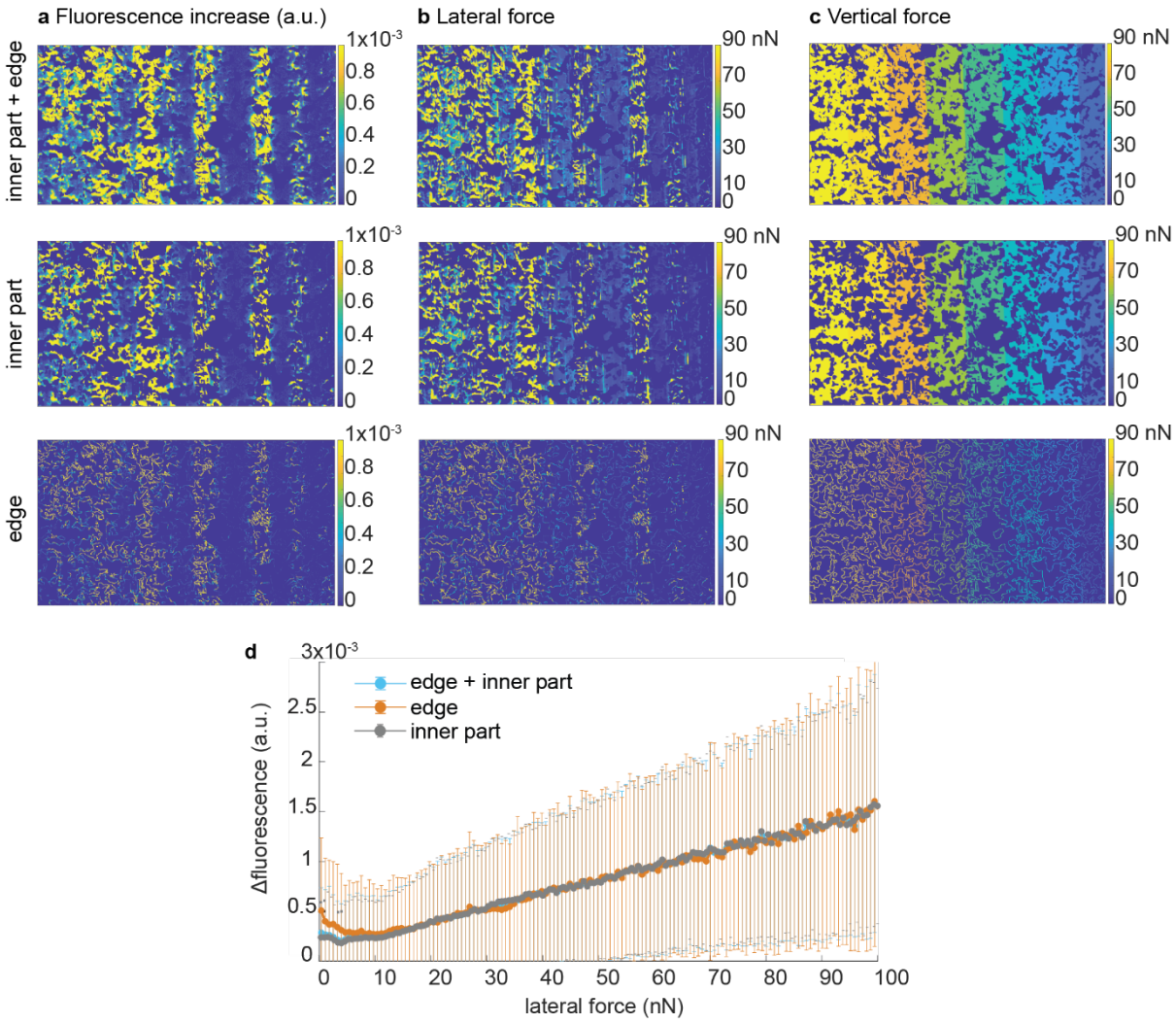


Figure S2: Separation of the inner part and the edge of the PDA islands made of PCDA in lateral force microscopy experiments. a, Fluorescence increase of PCDA LB films, b, AFM lateral force map and c, vertical force map. The images in the upper row show the whole scanned region without separation, the middle row shows only the inner parts of the PDA islands, while the edge regions of the PDA islands are displayed in the bottom row. The edge of a PDA island is defined as the 3 outermost pixels (145 nm). d, Fluorescence increase as a function of lateral force, separated for the inner part and edge regions. The data was collected to bins in the graph (d). The error bars show the standard deviation in each bin.

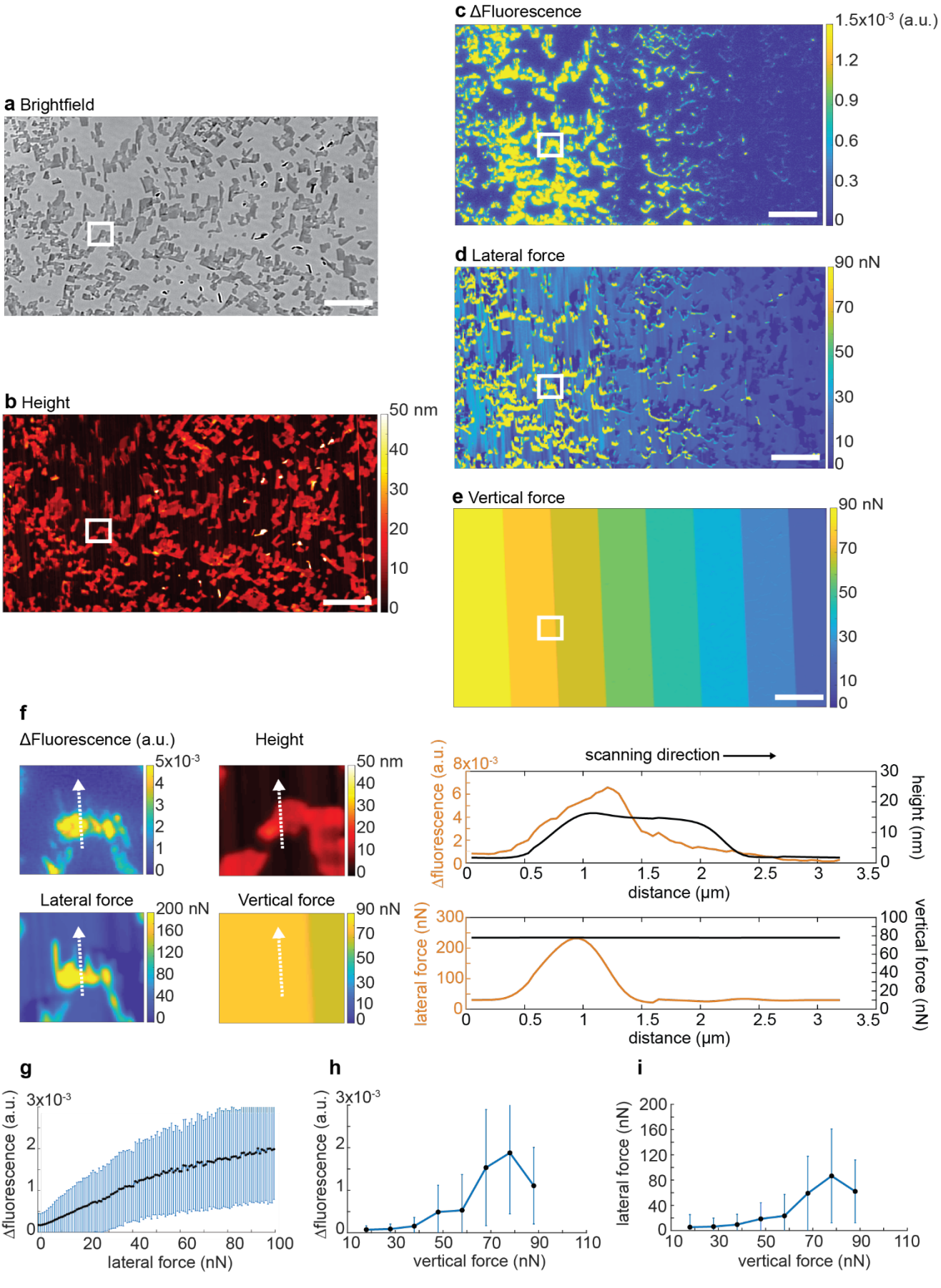
a Brightfield
b Height
50 nm
40
30
20
10
0
c ΔFluorescence
1.5x10-3 (a.u.)
1.2
0.9
0.6
0.3
0
d Lateral force
90 nN
70
50
30
10
0
e Vertical force
90 nN
70
50
30
10
0
f
ΔFluorescence (a.u.)
5x10-3
4
3
2
1
0
Height
50 nm
40
30
20
10
0
Lateral force
200 nN
160
120
80
40
0
Vertical force
90 nN
70
50
30
10
0
scanning direction
8x10-3
Δfluorescence (a.u.)
height (nm)
distance (μm)
lateral force (nN)
vertical force (nN)
distance (μm)
g
h
i
3x10-3
Δfluorescence (a.u.)
lateral force (nN)
3x10-3
Δfluorescence (a.u.)
vertical force (nN)
lateral force (nN)
vertical force (nN)

Figure S3. Characterization of 10,12-tricosadiynoic acid (TRCDA) Langmuir-Blodgett films by lateral force microscopy coupled with fluorescence microscopy. a, Brightfield microscopy image. b, AFM height. c, Fluorescence increase map. d, AFM lateral force map. e, AFM vertical force map. The scale bars show 10 µm. f, Zoom-in images of the white squares in images (b-e) and cross-sections along the white dotted arrows. Note that the white arrows in the zoom-in images showing the scanning direction are slightly tilted due to the alignment of the AFM images to optical images. These Δ fluorescence, Lateral force, Vertical force maps were correlated pixel by pixel and binned to generate plots in g-i; g, Fluorescence increase vs. lateral force, h, Fluorescence increase vs. vertical force, i, Lateral force as a function of the applied vertical force. The error bars show the standard deviation in each bin. Lateral force microscopy experiments were carried out in contact hover mode, with increasing setpoints (i.e. vertical force) after each 100 lines (20, 30, 40, 50, 60, 70, 80 and 90 nN) at 4 µm/s scanning speed. Areas of 50 x 100 µm were scanned at a resolution of 512 x 1024 pixel.

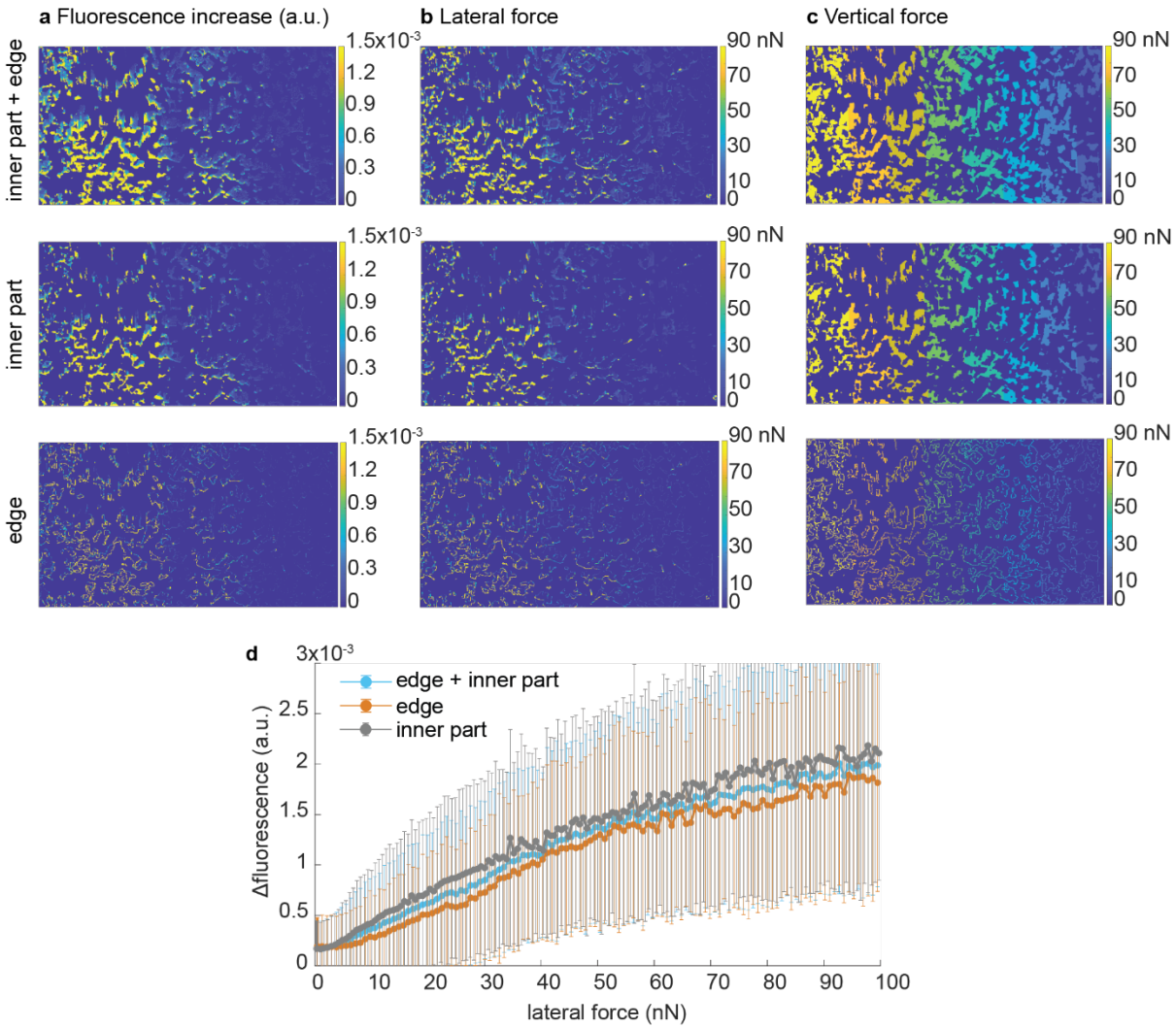


Figure S4: Separation of the inner part and edge of the PDA islands made of TRCDA in lateral force microscopy experiments. a, Fluorescence increase of TRCDA LB films, b, AFM lateral force map and c, vertical force map. The images in the upper row show the whole scanned region without separation, the middle row shows only the inner parts of the PDA islands, while the edge regions of the PDA islands are displayed in the bottom row. The edge of a PDA island is defined as the 3 outermost pixels (145 nm). d, Fluorescence increase as a function of lateral force, separated for the inner part and edge regions. The data was collected to bins in the graph (d). The error bars show the standard deviation in each bin.

Reference

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