Colloidal Covalent Organic Frameworks

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I. Materials and Instrumentation

Materials. 2,7-pyrenebis(boronic acid)$^1$ and 4,4'-diphenylbutadiynebis(boronic acid)$^2$ were prepared according to literature conditions. Reagents were purchased from commercial sources and used without further purification. CH$_3$CN, CH$_3$OH and toluene were purchased from commercial sources and purified using a custom-built alumina-column based solvent purification system. Other solvents were purchased from commercial sources and dried over activated 3Å molecular sieves.

Instrumentation.

Powder X-ray diffraction (PXRD) patterns were obtained on a Scintag Theta-Theta Powder X-Ray Diffractometer in reflectance Bragg-Brentano geometry employing Cu Kα line focused radiation at 2200 W (40 kV, 40 mA) power and equipped with a Ge crystal detector fitted with a 0.3 mm radiation entrance slit. Samples were mounted on zero background sample holders by dropping powders from a spatula and then leveling the sample surface with a glass microscope slide. No sample grinding or sieving was used prior to analysis. Crystallite size was determined by applying the Scherrer equation to the powder patterns using MDI JADE.

Small and wide angle X-ray scattering (SAXS/WAXS) measurements were performed at station G1 of the Cornell High Energy Synchrotron Source (CHESS), with an incident photon energy of 9.83 keV and sample-to-detector distances of 1.5 m for SAXS and 0.43 m for WAXS. Scattering patterns were recorded on two Pilatus 100k pixel array detectors (Dectris, Inc.). Patterns were corrected for incident photon flux and background-subtracted, then radially integrated using the Nika software package.$^3$ SAXS fits were performed to a spherical form factor using a maximum entropy method with the Irena software package.$^4$

Grazing incidence X-ray diffraction (GI-XRD) was performed at the G2 station at Cornell High Energy Synchrotron Source (CHESS) using a beam energy of 10.06 keV ($\lambda = 0.1232$ nm), selected using a single-crystal Be crystal
monochromator. Motorized slits were used to define a 0.2 × 3 (V×H) mm² beam, with a typical flux of 2×10¹⁰ photons s⁻¹. The data were collected using a 640-element 1D diode-array, of which each element incorporates its own pulse counting electronics capable of count rates of ~105 photons s⁻¹. A set of 0.1° Soller slits were used on the detector arm to define the in-plane resolution. Each data set was collected by scanning the detector with the sample stationary. The incidence angle, α, between the beam and sample surface was 0.175°. Axes labels Q┴ and Q∥ are defined using the GISAXS convention $Q_\perp = 4\pi/\lambda \sin(\delta/2)$ and $Q_\parallel = 4\pi/\lambda \sin(\nu/2)$, where δ and ν are the vertical and horizontal scattering angles, respectively. At $\alpha = \delta = 0$, ħ $Q_\parallel$ and ħ $Q_\perp$ (where ħ is Planck’s constant) are the components of momentum transfer parallel and perpendicular to the sample surface, respectively.

Dynamic light scattering measurements were conducted on a Malvern Zetasizer Nano-ZS equipped with a 4 mW He-Ne laser emitting at 633 nm. Measurements were optimized before each run and were taken in triplicate. The data were analyzed using Malvern Zetasizer Series Software v7.02.

Atomic force micrographs were taken on an Asylum MFP-3D-BIO operating in tapping mode and equipped with a Tap150Al-G Si tip with aluminum reflex coating using a set point of 550 mV. Colloid samples were prepared by dilution ten-fold and drop-casting onto freshly cleave d mica. Images were analyzed using the Gwyddion software package.

Scanning electron microscopy was performed on a LEO 1550 FESEM (Keck SEM) operating at 2.00 kV and a working distance of 3 – 4 mm with an aperture size of 20 μm. Samples were prepared by adsorption onto a silicon wafer, which was then attached to a flat aluminum platform sample holder. The sample was then placed directly into the instrument. No metal coating was applied.

Surface area measurements were conducted on a Micromeritics ASAP 2020 Accelerated Surface Area and Porosimetry Analyzer using samples degassed at 90 °C for 24 h and backfilled with N₂. N₂ isotherms were generated by incremental exposure to ultra high purity nitrogen up to 1 atm in a liquid

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nitrogen (77 K) bath and surface parameters were determined using BET adsorption models included in the instrument software (Micromeritics ASAP 2020 V4.00).

An FEI (FEI Company, Hillsboro, OR, USA) Tecnai G2 transmission electron microscope (TEM) with field emission gun (FEG), operating at 200 keV was used for the in situ VT-LCTEM experiments (Figure 5, Figure S11/S12/S13/S17, and Video S1/S2) and for dry-state TEM imaging of bulk-synthesized COF-5 colloids (Figure S15). The microscope was operated at a FEG emission current of ~150 µA. Calibrated beam current values between ~0.9 and 2.8 nA were used for all VT-LCTEM experiments (Table S3). TEM images were captured with a 2K x 2K Gatan Eagle CCD camera using FEI ES Vision software.

An FEI Tecnai Sphera TEM with LaB₆ gun, operating at 200 keV was used for the post mortem TEM characterization of the VT-LCTEM chips (Figure S14/S16). The microscope was operated at an emission current of ~4 µA (LaB₆ gun tip). TEM images were captured with a 2K x 2K Gatan CCD camera using Gatan Digital Micrograph image acquisition software (Roper Technologies, Sarasota, FL).

During the continuous irradiation VT-LCTEM experiments (Video S1/S2), the TEM camera was operated in continuous imaging mode (“search”) with a single frame exposure time of 0.3 s and image dimensions of 512 x 512 pixels (binning: 4). The images associated with the time-lapse VT-LCTEM experiments (Figure 5 and Figure S17), and all the conventional dry-state and post mortem TEM images were acquired with a 2 s exposure time and image dimensions of 2048 x 2048 pixels (binning: 1).

II. Experimental Protocols

General conditions for homogeneous synthesis of COF-5 colloid.

1,4-phenylenebis(boronic acid) (41 mg, 0.25 mmol) and CH₃OH (0.099 mL, 2.5 mmol) were combined with a dioxane / mesitylene solution (4:1 v/v, 16 mL). 2,3,6,7,10,11-hexahydroxytriphenylene (HHTP) (53 mg, 0.16 mmol) was added
and the solution was sonicated for 1 minute. The solution was then filtered (0.45 μm PTFE) to remove any trace residual particulate. Additional CH₃CN and/or dioxane/mesitylene blank solvent were added to reach a target % CH₃CN and [HHTP]₀. The solution was heated to 90 °C, for 20 hours, under atmospheric pressure.

**VT-LCTEM solution preparation: COF-5 precursor solution (55% CH₃CN).**

All solvents were either sieve-dried (dioxane and mesitylene) or fresh from the PNNL lab supply of dry solvent (acetonitrile and CH₃OH). Sieve-dried solvents were filtered with 0.45 μm PTFE syringe filters prior to measuring volumes and mixing. Oven-baked glassware was used in the preparation of the all solutions in this work. Solutions were sonicated for several seconds after all mixing steps involving the adding of powder (HHTP or PBBA) to solvent. Two slightly different solution preparation procedures were employed, both resulting in the same concentration of solvents and of HHTP and PBBA, but following different mixing steps.

**Procedure 1:** COF-5 55 vol% CH₃CN solution for “continuous irradiation” VT-LCTEM:

The entire COF-5 55 vol% CH₃CN precursor solution mixture is prepared ~40 hrs prior to being used for the LCTEM experiments (Figures S11-S13 and Videos S1/S2). ‘Part A’: 0.0305 g PBBA, 0.0403 g HHTP, 15.418 mL Dioxane/Mesitylene (4:1 vol%), 0.074 mL CH₃OH. ‘Part B’: Dioxane/Mesitylene (4:1 vol%), ‘Part C’: CH₃OH → **Final COF-5 55% Solution**: mix 1:0.8:2.2 (A:B:C vol%). The syringe pump used to load the liquid-cells was filled with ~300-800 μL of the Final Solution.

**Procedure 2:** COF 55 vol% CH₃CN solution for time-lapse VT-LCTEM experiments:

Two separate “monomer stock” solutions are prepared ~4 days prior to the VT LCTEM experiments. **PBBA stock (‘D’)** → 0.0195 g PBBA, 5 mL Dioxane/Mesitylene (4:1), 0.02464 mL CH₃OH. **HHTP stock (‘E’)** → 0.0261 g HHTP, 5 mL Dioxane/Mesitylene (4:1), 0.02464 mL CH₃OH. One
“solvent stock” solution was prepared ~24 h prior to the VT-LCTEM experiments. **Solvent stock (‘F’) → 1.425 mL CH$_3$CN, 0.518 mL Dioxane/Mesitylene (4:1).** To prepare the complete COF-5 55 growth solution → **1 part (D+E) is mixed with 3 parts F.** Just minutes prior to the VT-LCTEM experiment the complete COF-5 55 vol% CH$_3$CN growth solution is mixed (1:1:6 D/E/F) and then loaded into the liquid-cell using the external syringe (~300-800 µL of solution).

**VT-LCTEM heating protocol.**

**Time-lapse VT-LCTEM experiments (Figure 5 and Fig S17):**

With the holder at room temperature, all four corners of the liquid-cell are located at low-magnification (stage coordinates of each corner is saved), and one higher magnification image is acquired of each corner before the liquid cell is heated. With the e⁻ beam turned off, the liquid-cell solution temperature is ramped to 80 °C at a heating rate of 0.4 °C/s. The liquid-cell is held at 80 °C. The beam is then periodically turned on to acquire one snap-shot image of a corner region with the solution at 80 °C, starting after 10 minutes of reaching 80 °C (when drift has stabilized) and continuing for the following 4 hours. The beam is turned completely off between images, and the stage is moved to one of the other liquid-cell corners using the saved stage positions. At the new stage position, the beam was then turned on, the corner was properly centered, the image was focused, and one long-exposure image was acquired. This process was repeated over the entire duration of the VT-LCTEM time-lapse experiments. An electron dose rate of 0.66 e⁻/Å²s (~3.04 MGy/s) was used (Table S4).

**Continuous Irradiation VT-LCTEM Experiments (Figure S13 and Video S2):**

With the holder at room temperature, all four corners of the liquid-cell are located at low-magnification (stage coordinates of each corner are saved), and several higher magnification images are acquired of each corner before the liquid cell is heated. The beam is left on the entire time, at the dose rate conditions reported in Table S3. While acquiring continuous in situ video of one of the liquid-cell corners, the liquid-cell solution temperature is ramped to 90 °C at a heating rate
of 0.3 °C/s. Severe stage-drift occurs during the heat-ramp and during the first several minutes at 90 °C. The liquid cell is held at 90 °C. Periodically during the course of monitoring the corner region of interest, long-exposure LCTEM images (small pixel size, binning: 1) are acquired (Figure S11-S13). An electron dose rate of 0.85 e/Å²/s (~3.92 MGy/s) was used (Table S4).

Dry-state TEM analysis of bulk synthesis (COF-5 55% CH₃CN)

COF-5 55% CH₃CN growth solution was prepared using the same D/E/F (1:1:6) solution from the “zero” effective dose VT-LCTEM (procedure 2). The three stock solutions were mixed, and then the complete precursor solution was separated into 5 ependorfs (100 µL of complete precursor solution in each). The ependorfs were submerged into a heated water bath held at 90 °C. At various time points, starting at t = 5 min, one ependorf was removed from the heated water bath, and plunged into a room-temperature water bath at various time-points. Immediately after “quenching” an ependorf of heated solution, two 3 µL droplets was drop-cast onto a Formvar-carbon TEM grid (400 mesh Cu, Ted Pella, Inc., Redding, CA, USA). The droplets were dried with filter paper after ~1 min. The TEM grids prepared were loaded into the FEI Tecnai microscope using an FEI single-tilt holder. Images from solutions “frozen” at t = 15 min and t = 60 min are shown in Figure S15.

Liquid-cell assembly and set-up for LCTEM experiments.

The in situ LCTEM experiments were performed using a Protochips Inc. (Morrisville, NC) Poseidon Select liquid-cell TEM holder with on-chip heating capability (external power supply and computer controller). The inlet/outlet lines of the holder were initially dry when loading the liquid-cell chips. A pair of Protochips Poseidon Heating Chips with 50 nm thick SiNx membrane windows (50 µm x 400 µm and 550 µm x 20 µm lateral window dimensions respectively) were plasma cleaned for 60 s. For the continuous irradiation VT-LCTEM experiments (Figure S11-S13 and Video S1/S2) chip plasma cleaning was done using a South Bay Technology Inc. PC2000 plasma cleaner (San Clemente, CA)
with a 220 mbar pressure 1:1 Ar/O\textsubscript{2} gas blend, operating at forward power: 13 and reflected power: 4; for the time-lapse VT-LCTEM experiments, chip plasma cleaning was done using a Harrick Plasma Inc. (Ithaca, NY) PDC-32G-2 plasma cleaner operating at “low” RF power mode with a 9:1 Ar/O\textsubscript{2} gas blend. One of the two chips used to assemble each liquid-cell had the micro-fabricated on-chip heating element (“heating chip” \textit{Protochips EFT-45ZZ}), and the other chip was a “spacer chip” \textit{(Protochips EPB-52DF)} with 150 nm tall SU-8 spacer channel to direct solution flow over the window area. The spacer chip was loaded (membrane up) into the holder tip, and is oriented with spacer flow channel perpendicular to the holder shaft. The heating chip was placed down (membrane facing down) over the spacer chip. The windows of the two chips were crossed, so that the viewing area (e\textsuperscript{-} transmitting) was a 50 µm x 20 µm rectangle. The cover-plate of the tip was put into place, and the screws were tightened with a torque screwdriver.

The window integrity and O-ring seal of the liquid-cell was checked by pumping the holder down to vacuum (8 x 10\textsuperscript{-6} mbar) in a “pre-pump” vacuum chamber designed for FEI TEM holders. As the holder tip was being pumped to high vacuum in the pre-pump chamber, the COF-5 precursor solution (either prepared by procedure 1 or procedure 2) was flowed into the holder using the holder’s Peek tubing inlet line, at a flow rate of 8 µL/min via syringe pump. Flow was continued for ~3 min after the first droplets of liquid began coming out the outlet line. At this point the pre-pump chamber has reached maximum frequency and ~8 x 10\textsuperscript{-6} mbar should be achieved for a properly sealed liquid-cell. The integrity of the windows were also visually inspected for any cracks/leaks and for the signs of being filled with air pockets rather than completely filled with solution. If the liquid-cell passed the visual inspection at high vacuum and liquid flow, the vacuum of the pre-pump chamber was released to atmosphere. The inlet flow of solution was then stopped, and back of inlet/outlet lines were sealed to prevent drying during the LCTEM experiment. The holder was inserted into the TEM to begin the \textit{in situ} observations and initiate the on-chip liquid heating. The window
region of the liquid-cell was found within ~15 minutes of preparing the COF-5 precursor solutions in VT-LCTEM experiments.

**LCTEM e⁻ dose conditions.**

Prior to the performing LCTEM experiments, the beam current at the sample plane of the PNNL FEI Tecnai was directly measured using a Gatan double-tilt holder with built-in faraday cup. The accuracy of the beam current value from the small-screen inside the microscope was calibrated to the faraday cup measurement to determine the error factor in the beam current for that Tecnai. The error-factor relationship is linear over the range of relevant beam currents. At the start of each LCTEM experiment, the beam current value from the small screen was recorded for several the several spot sizes (3-6 in TEM mode) that might be used during the experiment. The LCTEM holder was slightly removed from the microscope column so that the beam was passing through vacuum. The calibrated beam current value (in nA) was then used to calculate to the dose rate in e⁻/Å²/s for each LCTEM experiment. The e⁻ beam diameter was measured for each magnification used in the LCTEM experiments. The dose rates in Gy/s were calculated according to Ref. 5, using a Total Stopping Power value for liquid CH₃OH and 200 keV electrons (NIST ESTAR database). A table of the calibrated beam current values and the calculated dose rate for all the LCTEM experiments is listed in Table S3, and the total cumulative dose for the all the time-lapse VT-LCTEM image-series are listed in Table S4. Similar dose rates (same order of magnitude) to those use during the continuous irradiation CT-LCTEM experiments were used during the acquisition of the time-lapse VT-LCTEM images (Figure 5 and Figure S17), but the beam exposure time was kept very short (~30 s to align of interest in the field of view, adjust focus, and acquire each image), with long intervals between beam exposures where the beam was completely off.

To start all the LCTEM experiments (continuous irradiation and time-lapse), general focusing and locating of the corners of the liquid-cell was done at low magnification, minimizing beam exposure and cumulative dose (dose rate at
least 1-order of magnitude lower than those used during the prolonged exposure VT-LCTEM videos). The beam was shut off completely between images in the time-lapse VT-LCTEM experiments, but was left continuously on in the continuous irradiation experiments.

**LCTEM live video acquisition.**
The raw LCTEM videos (Videos S1/S2) were acquired using Camtasia Studio 7 Recorder screen capture software (TechSmith Corporation, Okemos, MI, USA), at a frame capture rate of 4 frame/s. The screen capture software was directed at the FEI ES Vision software window that was continuously acquiring the TEM images from the camera at native resolution (512 x 512 pixels, binning 4, exposure-time 0.3 s). The LCTEM images that are displayed in Figures S11-13, are ‘acquire’ images (2 second exposure time, 2048 x 2048, binning 1). The supporting video files (Video S1/S2) were created using Camtasia Studio 7 video editing software to truncate the length of the raw LCTEM video clips to the desired starting and ending time-points, and to adjust the video playback speed (supporting videos have been sped up from real-time).

**Image alignment of time-lapse VT-LCTEM image-series.**
Each time-lapse series of *in situ* VT-LCTEM images (Figure 5 and Figure S17) was assembled into an image-stack using Fiji software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016). The images in the stack were aligned using the “Template Matching” cross-correlation plugin (“align slices in stack”), with the window corner selected as the feature used as the ROI to align each image-stack. The precision of the stack alignment was verified by visual inspection. The aligned time-lapse image-stacks were used as the input for quantitative MOTA analysis.

**Multi-Object Particle Tracking Analysis (MOTA).**
An automated multi-object tracking algorithm was developed and applied to the raw LCTEM videos to automatically tracks individual particles for each image of the time-series.\textsuperscript{7,8} The automated algorithm first detects potential particles in the first image that they appear in the time-lapse image-series using image segmentation algorithm,\textsuperscript{7} and associates any particle motion/aggregation over time (in subsequent images) based on the spatial proximity and geometrical similarities of the detected particles;\textsuperscript{8} during the process, most faulty detections are filtered out. Additional faulty detections were manually filtered out by thoroughly reviewing the data output for irregularities. In the MOTA analysis; \(i\) denotes the index number for marking the identity of a particle, and \(t\) denotes the time index. The main output of the tracking algorithm is the silhouette image of particle \(i\) observed at time \(t\) in the form of a set of all image pixel locations on the interior of the particle, \(\{(x_n^{(i,t)}, y_n^{(i,t)}); n = 1, \ldots, N_{it}\}\). Let \(\mathbf{X}_{it}\) denote the \(N_{it} \times 2\) matrix of the pixel locations. We computed the spatial location of each particle’s center of mass and the interior area of each particle by taking the column mean and the row size of the matrix.

To threshold the objects in each time-lapse VT-LCTEM image, we constrain the object shape to be elliptical (perfectly smooth, rounded elliptical perimeters for all particles), \textit{i.e.} a size-fitted elliptical mask is applied to each object. This elliptical-masking method was employed due to the relatively low contrast of the COF particles against the liquid-cell background. The particle area measurements used to create the average particle size measurements in Figure 5 and Figure S17 are those of the ellipses fitted to best match the thresholded object’s cross-sectional area.

**Quantitative analysis of MOTA data (VT-LCTEM particle-size measurement).**

The data output from the MOTA analysis of the aligned time-lapse VT-LCTEM image-series (1. object center of mass \(x,y\)-position, and 2. ellipse-masked area for each object in each image) was manually inspected for proper thresholding and ellipse-masking of the objects, by visually comparing raw images with the corresponding ellipse-masked MOTA image). Overlapping particles identified
during manual inspection were removed from the MOTA data sets that were then used to compute average individual-particle size in each frame. Using Microsoft Excel software (Microsoft Corp., Redmond, CA, CA), average particle size and standard error (above and bellow the mean average) were calculated for each frame in the time-lapse VT-LCTEM image stacks. Matlab software (MathWorks Inc., Boston, MA, USA) was used to plot the average particle size (average error bars) vs. time held at 80 °C for each time-lapse VT-LCTEM experiment (Figure 5 and Figure S17).

Post mortem TEM characterization of used VT-LCTEM chips.

Due to the size/geometry of the Protochips heating chips, which are much too large to fit in conventional TEM holders, only the smaller “spacer” chips could be investigated post mortem by conventional TEM. The spacer chips were loaded into an FEI single-tilt holder for post mortem TEM characterization. In some cases, evidence of COF growth was found on the surface of the spacer chips, in other cases no nanostructures were found on the spacer chip. For the chips used in VT-LCTEM heating experiments with prolonged continuous e-beam irradiation (Figure S11-S13), the location on the SiNx window where the LCTEM videos were acquired (location of direct beam irradiation) could be clearly identified by the characteristic circle of deposited cubic facetted nanocrystals with diameter close to that of the beam diameter during LCTEM illumination (Figure S14D/F).

Formation of free standing COF film

COF-5 colloid solution (2 mM [HHTP]0, 10 mL) was heated to 90 °C in an uncapped scintillation vial exposed to atmosphere. Upon complete evaporation of the solvent mixture, the vial was cooled to room temperature.
III. Additional Characterization

A. Additive Solvent Survey

Figure S1. (A) Tyndall effect demonstration of colloidal COF synthesized in the presence of acetonitrile (right) and the initial monomer solution prior to heating (left). (B) COF-5 formation as a function of additive solvent (4mM [HHTP]0, 1.5 eq. PBBA, 15 eq. CH3OH, 90 °C, 24 h) Solvent: 50 vol% additive, 50% 4:1 dioxane:mesitylene.

Table S1. COF-5 precipitate yield and crystallite size as a function of additive solvent

| Additive Solvent            | Yield (%) | Crystallite Size (nm) |
|-----------------------------|-----------|-----------------------|
| 4:1 dioxane:mesitylene      | 78        | 19 (1)                |
| Toluene                     | 89        | 21 (1)                |
| THF                         | 72        | 20 (1)                |
| DMF                         | 63        | 25 (2)                |
| CHCl3                       | 86        | 24 (1)                |
| CH2Cl2                      | 87        | 23 (2)                |
B. SAXS/WAXS

**Figure S2.** SAXS particle size distribution and full width at half max (FWHM) of the \<100\> diffraction peak of COF-5 colloid formation over time.

**Figure S3.** Stacked SAXS of *in situ* COF-5 colloid formation over time.
Figure S4. Example fitting for SAXS particle size of \textit{in situ} COF-5 colloid formation.

Figure S5. SAXS/WAXS of \textit{in situ} DPB-HHTP COF colloid formation over time, with inset of the highlighted <100> diffraction peak (growth conditions: 2 mM [HHTP]$_5$, 1.5 equiv DPB, 15 equiv CH$_3$OH, solvent 55 vol\% CH$_3$CN, 90 °C).
C. Dynamic Light Scattering

Table S2. Colloidal COF-5 DLS particle data

| % CH$_3$CN | Initial 20 h growth (90 °C) | After 1 month at 25 °C |
|------------|-----------------------------|-------------------------|
|            | Z-avg (nm) | PDI | Intensity Mean (nm) | Number Mean (nm) | Z-avg (nm) | PDI | Intensity Mean (nm) | Number Mean (nm) |
| 15         | 232        | 0.014 | 239               | 220               | 230        | 0.022 | 240               | 218               |
| 35         | 109        | 0.103 | 122               | 74                | 110        | 0.092 | 122               | 76                |
| 55         | 62         | 0.158 | 73                | 41                | 61         | 0.161 | 72                | 39                |
| 75         | 46         | 0.185 | 56                | 30                | 43         | 0.170 | 52                | 31                |
| 95         | 50         | 0.115 | 57                | 33                | 49         | 0.111 | 56                | 32                |

Figure S6. Colloidal COF-5 DLS Z-avg size after post-synthetic modification of the solvent % CH$_3$CN. Where error bars correspond to the polydispersity width. Colloids were exposed to the new solvent conditions for 24 hours, both at room temperature and 90 °C.
D. Atomic Force Microscopy

Figure S7. AFM of COF-5 colloids prepared at different solvent concentrations of CH$_3$CN, and drop-cast onto freshly cleaved mica.
Figure S8. Particle height distribution histogram as a function of % CH$_3$CN
E. N₂ Isotherm and BET surface area determination

Figure S9. N₂ adsorption isotherm (77 K) and surface area data analysis of COF-5 free standing films.
F. Powder X-ray diffraction

Figure S10. Powder X-ray diffraction of COF-5 free standing thin films.
G. VT-LCTEM and post mortem TEM.

**e⁻ Beam Sensitivity and Damage Tests:**

At 27 °C, COF-5 precursor solution (55 vol% CH₃CN) exhibits only minor damage during 19 minutes of continuous “low-dose” LCTEM illumination (where dose rate = 0.33 e⁻/Å²s or 1.52 MGy/s, see Video S1 and Figure S11), with no gross nanostructure nucleation and no growth occurring. However, signs of solvent degradation are clearly observed, reminiscent of the e-beam radiolysis degradation of organic electrolyte solutions.⁹ In regions of the liquid-cell, which did not experience prolonged beam illumination no nanomaterial nucleation/growth or solvent degradation is detected when the liquid temperature is held at 27 °C for 20 minutes (Figure S12). LCTEM, as with scattering experiments, reveals that the polymerization mixture does not yield COF nanostructures at room temperature under the timescales observed. Therefore, we deployed on-chip temperature control, to acquire continuous VT-LCTEM video of the COF 55% CH₃CN growth solution as the liquid was heated at a rate of 0.3 °C/s over approximately 4 minutes to 90 °C where it was held (Figure S13 and Video S2). Nanostructure formation is detectable during the ramp to 90 °C, with video data revealing a time series of nanostructure growth starting 1.5 min after the solution temperature has stabilized at 90 °C (Figure S13B-J).

Nanoparticle seeds nucleated over the entire region of view, which continue to grow individually, without significant aggregation or coalescence. The uniform, radial growth is reminiscent to that of ZIF-8 metal-organic frameworks (MOFs), which has been attributed to surface-limited growth of seed particles during monomer/ligand attachment from solution.¹⁰

Nanomaterial growth is not limited to the region directly irradiated by the e⁻ beam, but rather extends into the bulk of the liquid-cell, away from the corner region, where liquid thickness is large and image resolution/contrast is lost (Figure S13K). Exclusively in the regions exposed to prolonged e⁻ beam illumination, many of the nanoparticles that have formed are highly-faceted (cubic), which we identify as boron-oxide (B₂O₃)¹¹ crystals by post mortem diffraction analysis of the used liquid-cell chips (Figure S14). We find that in
areas outside the region directly under e\textsuperscript{−} beam illumination (beyond the ring of dense material growth in Figure S13), particle morphology is consistent with that of the bulk-grown COF nanoparticles (Figure S15). Clearly, the e\textsuperscript{−} beam irradiation causes sufficient radiolidic damage at elevated temperatures to alter the nucleation and growth mechanisms, and the structure/morphology of the nanomaterials that form, likely by influencing the solution chemistry and concentration of free-radical species. More exploration into lower dose LCTEM imaging methods is needed to enable VT-LCTEM live-video, as it is likely that the characteristic increased beam-sensitivity of these COF solutions exhibit at elevated temperatures extends to other systems of interest for VT-LCTEM analysis.

![Figure S11](image)

**Figure S11.** *In situ* LCTEM images of one corner of the liquid-cell 19 minutes apart, while the COF 55% CH\textsubscript{3}CN growth solution is held at 27 °C (Video S1). This region has been continually irradiated by the e- beam during the 19 minutes that separate the two frames (dose rate: 0.33 e/Å\textsuperscript{2}s or 1.52 MGy/s). Several pre-formed nanostructures are present in this region of the liquid-cell prior to prolonged irradiation, which do not grow or degrade significantly during irradiation. Evidence of some solvent degradation is present in the increased “patchy” background contrast (shadowy features covering entire frame after 19 min of irradiation). The pieces of larger “nano”-material are distributed over the surface of the silicon nitride membrane (left) are likely ‘dust’ particles commonly found in LCTEM experiments due to chip handling during liquid-cell assembly. No sub-100 nm COF colloids are detected in the liquid cell at room temperature.
Figure S12. *In situ* LCTEM images of one corner of the liquid-cell 20 minutes apart, while the COF 55% CH$_3$CN growth solution is held at 27 °C. The region is not exposed to e- beam between images (“zero” effective cumulative does) and is ~40 µm away from the region under prolonged irradiation (i.e. region of Figure S11 and Video S1). No sub-100 nm COF colloids are detected in the liquid cell at room temperature in regions not exposed to prolonged e- beam irradiation.

![Image of LCTEM images](image-url)

Figure S13. (A) LCTEM image of COF-5 precursor solution at 27 °C, before solution is heated. No COF nanostructures are detected at room temperature. (B-K) Time series of bright-field VT-LCTEM images as the COF 55% CH$_3$CN growth solution is held at 90 °C while under continuous e- beam irradiation (dose rate: 0.85 e/Å$^2$/s or ~3.92 MGy). Region of B-J frames are indicated as red squares in A and K. The region of the liquid-cell that was directly illuminated by the TEM beam during the continuous-irradiation VT-LCTEM experiment is roughly outlined by the ring of dense nanomaterial growth in K. Video S2 is the corresponding video for this VT-LCTEM experiment.
Figure S14. *Post mortem* TEM characterization of liquid-cell chips used during the “continuous illumination” VT-LCTEM experiment shown in Figure S13 (Video S2). (A-C) BF TEM images of the window surface in several locations where there was no e⁻ beam illumination during the VT-LCTEM experiment (these regions are tens of microns away from the region in Figure S13) Rounded colloidal nanoparticles are found, very similar to those observed in the dry-state TEM images of samples grown by bulk-solution synthesis (Figure S15). (D-F) BF TEM images of regions that were directly imaged (prolonged e⁻ beam illumination) during the VT-LCTEM experiment in Figure S13 (Video S2). The predominant structures are highly-facetted cubic nanoparticles, but there are also many smaller, rounded nanoparticles (E). (G) Selected area diffraction pattern of the central region of frame F. Ring spacings correspond to periodic lattice spacings of: 2.38 Å, 2.06 Å, 1.46 Å, 1.25 Å, 1.19 Å, and match the trirhahedra structure of crystalline B₂O₃,¹¹ which is one likely radiolysis product from e⁻ beam irradiation of the boronate-monomer/solvent COF growth solution. (H) BF TEM image, and (I) corresponding dark field (DF) TEM image of the center region of frame F (one of the predominant inner-ring diffraction spot is selected with the objective aperture). The Nanocrystals in frame I of bright intensity (white) are on on-axis for the diffraction spot selected by the objective aperture, demonstrating the high-degree of crystallinity in the B₂O₃ nanocrystals.
Figure S15. Dry-state TEM images of COF-5 nanoparticles grown by bulk-solution synthesis of the 55% CH$_3$CN precursor solution (90 °C). Left and center frames are of the growth solution taken at the 15-minute time point at 90 °C, and the right frame is of the growth solution taken at the 60-minute time point at 90 °C. Solution contains small and rounded colloidal nanoparticles of ~20-30 nm diameters.

Figure S16. Post mortem TEM characterization of the liquid-cell chips used during the time-lapse VT-LCTEM experiment (COF 55% growth solution) shown in Figure 5. Consistent with the in situ LCTEM images from these heating experiments (Figure 5 and Figure S17), the nanoparticles found on the dried chips are extremely uniform in size and shape, and remain discrete particles, not large aggregates. No highly-faceted cubic nanocrystals are found on these chips (no evidence of B$_2$O$_3$ crystallites), which suggests minimal beam-related artifacts in VT-LCTEM data when the time-lapse method has been employed.
Figure S17a. (A) Time series of bright-field VT-LCTEM images as the COF 55% CH₃CN growth solution is held at 80 °C. Same VT-LCTEM experiment as Figure 5 and Figure S17b,c (which are the other three window-corners of this liquid-cell). First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.

Figure S17b. (A) Time series of bright-field VT-LCTEM images as the COF 55% CH₃CN growth solution is held at 80 °C. Same VT-LCTEM experiment as Figure 5 and Figure S17a,c (which are the other three window-corners of this liquid-cell). First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.
Figure S17c. (A) Time series of bright-field VT-LCTEM images as the COF 55% CH$_3$CN growth solution is held at 80 °C. Same VT-LCTEM experiment as Figure 5 and Figure S17a,b (which are the other three window-corners of this liquid-cell). First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.

Figure S17d: (A) Time series of bright-field LCTEM images as the COF 55% CH$_3$CN growth solution is heated. Different VT-LCTEM experiment as Figure 5 and Figure S17a,b,c, but same VT-LCTEM experiment as Figure S17e,f. First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.
Figure S17e. (A) Time series of bright-field LCTEM images as the COF 55% CH₃CN growth solution is heated. Different VT-LCTEM experiment as Figure 5 and Figure S17a,b,c, but same VT-LCTEM experiment as Figure S17d,f. First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.

Figure S17f. (A) Time series of bright-field LCTEM images as the COF 55% CH₃CN growth solution is heated. Different VT-LCTEM experiment as Figure 5 and Figure S17a,b,c, but same VT-LCTEM experiment as Figure S17d,e. First frame is the COF precursor solution at room temperature (no nanostructures), the following frames are of the ROI once the solution has been heated to 80 °C using on-chip in situ variable-temperature control. (B) Discrete particle diameter, as measured from each LCTEM image using multi-object image analysis. Average particle size is marked by black dots and standard error is marked by red dots.

Supporting Video Captions:
**Video S1.** *in situ* LCTEM continuous e⁻ beam irradiation experiment of the COF 55% CH₃CN growth solution at room temperature (27 °C), shown as a time-series in Figure S11. Dose rate is ~0.33 e⁻/Å²s or ~1.52 MGy. The TEM CCD camera is operating at 3.33 frames/s (0.3 s exposure time) and 512x512 frame size (binning 4). The video playback has been sped up to x50 speed, and is ~19.33 min duration in real-time. No nanostructure growth is detected at these imaging conditions at room temperature, but gradual degradation of the solvent is evident (most clear as the patching dark intensity features in the long exposure image in Figure S11 right).

**Video S2.** *in situ* VT-LCTEM continuous e⁻ beam irradiation experiment of the COF 55% CH₃CN growth solution as the liquid-cell is heated to, and held at 90 °C (heating rate 0.3 °C/s), shown as a time-series in Figure S13. Dose rate is ~0.85 e⁻/Å²s or ~3.92 MGy. The TEM CCD camera is operating at 3.33 frames/s (0.3 s exposure time) and 512x512 frame size (binning 4). The video playback has been sped up to x30 speed, and is ~15.15 min duration in real-time. Nanostructure nucleation can be detected within the first couple minutes at 90 °C, but due to stage drift, image/video resolution is too poor for quantitative analysis. Once drift has settled, image quality improves, and a mixture of cubic-facetted nanoparticles and spherical nanoparticles cover the viewing area. Both morphologies of nanoparticles grow individually over time, with the more rapid growth in the faceted particles, which become larger and dominate the field of view at the end of the experiment. *Post mortem* analysis (Figure S14) confirms that the cubic-facetted nanoparticles are not COF-5 structures, but are boron oxide (B₂O₃) crystals, likely the product of e⁻ beam radiolysis related artifacts on the growth processes for this precursor solution at 90 °C.

**Supporting Tables:**

**Table S3:** TEM imaging conditions used for all the LCTEM experiments. Dose rate in MGy/s was calculated according to ref. 5 using a total stopping power (S) value for CH₃OH (a reasonable approximation for the solvent mixture of the COF precursor solution, as most organic solvents have very similar S-values. S-value for liquid-H₂O is also very similar).

| Experiment (video or time-series) | Calibrated TEM beam current (nA) | Beam diameter (µm) | Dose rate (e⁻ /Å²s) | Dose rate (MGy/s) |
|----------------------------------|---------------------------------|--------------------|---------------------|------------------|
| Video S1/Figure S11              | 1.064                           | 8                  | 0.33                | 1.52             |
| Video S2/Figure S13              | 2.75                            | 8                  | 0.85                | 3.92             |
| Figure 5/Figure S17              | 0.937                           | 5.3                | 0.66                | 3.04             |

**Table S4.** Cumulative electron-doses for the time-lapse VT-LCTEM experiments.
| Time-lapse VT-LCTEM experiment | Approximate total time of irradiation over entire time-lapse (s) | Approximate cumulative dose \( (\text{e}^+\text{Å}^2) \) | Approximate cumulative dose \( (\text{MGy}) \) |
|-------------------------------|------------------------------------------------|---------------------------------|---------------------------------|
| Figure 5                     | 310                                           | 205                             | 924                             |
| Figure S17a                   | 400                                           | 264                             | 1192                            |
| Figure S17b                   | 280                                           | 185                             | 834                             |
| Figure S17c                   | 430                                           | 284                             | 1281                            |
| Figure S17d                   | 190                                           | 125                             | 566                             |
| Figure S17e                   | 190                                           | 125                             | 566                             |
| Figure S17f                   | 340                                           | 224                             | 1013                            |

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